# 9 New Technologies and Tissue Repair and Regeneration (1): Stem Cells, Tissue Engineering, and 3D Technology

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## 9.1 Tissue Engineering and 3D Technology

Since its inception, tissue engineering technology has made significant progress in many fields such as biomedicine, such as the separation of new cell sources and seed cells, the design and synthesis of high biomimetic biomaterial scaffolds, the invention of new drugs and delivery systems, and the development of flux bio-manufacturing technology. Such results have led to the rapid development of some innovative clinical treatment strategies; especially, the repair and regeneration of tissue and organ damage have achieved satisfactory clinical results. At present, tissue engineering and regenerative medicine strategies can be broadly classified into three categories: cell-based therapeutic strategies; stent-therapeutic strategies with acellular scaffolds or seeded cells; and cell-loaded structures or matrix complexes.

Specifically, cell-based therapies are allogeneic or xenogeneic cells taken from patients (autologous) or donors (same species, xenogeneic), after further processing (e.g., cell sorting and in vitro expansion) into the lesion. This therapy is relatively simple, but faces problems such as how to maintain cell activity and colonization. Therefore, a new strategy for immobilizing specific cells in biomaterial carrier scaffolds to enhance colonization and viability has emerged. Depending on the degree of damage and the biomechanical properties of the tissue, different scaffold implant materials can be selected to provide a specific environment for the cells, guiding the penetration, colonization, attachment, and proliferation of seed cells or host cells, promoting the synthesis of new extracellular matrix, thereby improving clinical efficacy. Our research team loaded gelatin microspheres containing sweat gland cells into tissue-engineered skin. It can form a full-thick skin with sweat gland structure in vitro, promote complete repair of skin in vivo, and provide a new strategy for clinical treatment of wound.

Currently, there are generally two ways to perform organ transplantation using cell 3D printing technology. Taking the liver as an example, one is to first scan the shape of the patient's liver, insert the biomaterial into the body, and then implant the cells, using the internal environment to grow the liver with sufficient nutrient solution; the species is printed directly in vitro and transplanted into the patient. The second method is more scientific and the effect will be more ideal, but to achieve a high technical content, there is still a certain distance from the application. Moreover, even if the 3D-printed artificial organs function normally in vitro, it is currently unknown whether they can be operated and what are the adverse reactions once implanted in the body. In addition, the nutrient absorption and excretion of organs after transplantation is the process of gradually repairing, adapting, and replacing the old organs in the body. If the organ support system can be constructed and studied first, the rejection reaction and relevant risk after organ implantation in the human body will be reduced [1].

On the other hand, before the real maturity of 3D printing organ technology, a series of high costs such as research and development and market development will become high-end consumption. Especially in China, 3D printing technology is still in the initial stage of development, and there are still bottlenecks in technology. If the type and performance of the material are limited, the efficiency of forming needs to be

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X. Fu (ed.), *Regenerative Medicine in China*, https://doi.org/10.1007/978-981-16-1182-7_9

further improved, and there is an urgent need for reinforcement in the size, precision, and stability of the process. Moreover, China's 3D printing consumables mainly rely on foreign imports. Excessive material costs may be one of the reasons hindering development, which will greatly reduce the cost performance of 3D printing technology, and to some extent weaken the market's emphasis on this technology. Therefore, at this stage, the investment in 3D printing in the field of biomedicine should focus on strengthening innovation and research and development, technology introduction and reserve, especially paying attention to the construction and maintenance of independent intellectual property rights, and strive to occupy a favorable position in the future market competition.

Currently, the biomedical field has become one of the most popular areas for 3D printing applications. In 2012, the output value of 3D printing technology in this field accounted for 16.4% of the global output value. Most of the applications focused on prosthetic manufacturing, orthodontics, and restoration. By the end of 2013, there were as many as 30,000 successful cases of using titanium 3D printing to make titanium human bones. In addition, with the advancement of technology, cost reduction, aging of the population, and the small number of donated organs, the industry is generally optimistic about the development prospects of 3D bioprinting technology in the field of regenerative medicine, especially organ transplantation. With the continuous advancement of technology, the technology of applying 3D printing to tissue and organ transplantation is not only at the theoretical level. Moreover, each person's body structure and pathological conditions are unique and differentiated; when 3D printing is combined with medical image modeling and simulation techniques, a great driving effect can be generated in the manufacture of artificial prostheses, implants, and artificial tissues. I believe that in the near future, scientists will use a research result to prove the possibility of "organized transplants." However, the regulatory mechanism for 3D printed biological organs has not yet been established, and its classification and auditing standards in biomedical products are in a state of ambiguity. These all indicate that in addition to the technical development of 3D bioprinting, there is still a long way to go to establish a review mechanism for escorting this huge future emerging industry [2, 3].

## 9.2 Stem Cells and Related Technologies and Skin Repair and Regeneration

Stem cells are one of the most rapid advances in basic research and clinical applications of life sciences in recent years. Stem cells and related technologies have shown great potential for cell therapy, tissue and organ repair, developmental biology, and pharmacology. Since stem cells have the characteristics of regeneration and differentiation, stem cells implanted in the body or tissues constructed from stem cells in vitro can be implanted into the body to differentiate and regenerate damaged organs. At present, domestic and foreign institutions have successfully tried various tissues and organ regenerations using stem cells. For example, scientists use bone marrow mesenchymal stem cells and umbilical cord mesenchymal stem cells to differentiate into muscle cells, nerve cells, and sweat gland cells in vitro and in vivo, and bring hope for the treatment of large-area trauma, such as muscle atrophy, brain atrophy, Parkinson's disease, and other degenerative diseases and burn wounds; embryonic stem cells, neural stem cells, epidermal stem cells, and dermal papilla cells are isolated from developing or adult embryonic stem cell tissues, and induced pluripotent stem cells can be obtained in vitro, and implanted in vitro after expansion and culture. The site is differentiated into neurons, astrocytes, oligodendrocytes, epidermal cells, hair follicles, sebaceous glands, and sweat glands, providing new strategies for nerve tissue and wound repair.

At present, stem cells used in stem cell therapy and tissue engineering are self-stem cells, and it is difficult to industrialize regenerative medicine results. The establishment of low-immunogenic stem cells to achieve universal seed cells across individual applications is a need to achieve tissue engineering industrialization. In addition, the safety of stem cell therapy is an important prerequisite for clinical application of stem cells. Methods for stem cells to enter the body include direct intratissue injection, as well as intravascular injection or by tissue engineering scaffold-based transport. Safe, effective, and practical route of administration is the key to the success of stem cell regeneration therapy, mainly to ensure the effective cell dose and targeting of tissue regeneration, and it is hoped that it will be less distributed in non-targeted areas and spread the blood source. The danger is minimized.

The skin is the largest in the human body, and it is also the first organ damaged after wounding (burning, war). After mild injury (burning, war), the skin and its accessory cells are destroyed, and their repair and regeneration often use their uninjured parts as a template to achieve complete repair through proliferation and differentiation. However, in the case of large-scale wounds (burning, war) in the whole layer, due to the complete destruction of the skin, the skin and its accessory cells cannot completely rely on the division, proliferation, and differentiation of the stem cells to rebuild their complex structures, thereby making the skin and the attachment regeneration difficult, and eventually there will be two aspects of healing problems, namely the formation of chronic difficult-to-heal wounds (commonly known as chronic ulcers) or excessive repair of hypertrophic scars and keloids, not only for the physical and psychological aspects of patients' serious obstacles, but also have a serious impact on the quality of life and work in the later period. The patients suffer a lot from the damage of sweat gland cells. Sweating is the main way for the

body to regulate body temperature. The sweat glands are all over the body. It is difficult to regenerate and leads to a very low quality of life. It is even life-threatening in hot climates. As the most abundant gland of the body, the sweat gland can form a layered epidermis under certain conditions, and its role in skin damage repair is more important than epidermal cells. Our team used inactivated sweat gland cells to coculture with MSC and added sweat gland induction medium to induce differentiation of mesenchymal stem cells into sweat gland cells, overexpressing Eda in MSC, reprograming these two cells into sweat gland cells, and participate in skin repair by transferring NF-κB and Lef1 into fibroblasts. Liang et al. induced the differentiation of amniotic fluid stem cells into sweat gland cells by using sweat gland culture medium. Further experiments and exploration are needed to apply the above various sweat gland cell regeneration methods to the clinic. At present, the commonly used repair methods mainly include autologous free skin graft, flap transplantation, allogeneic skin, and xenograft. Autologous skin grafting is the best method for wound defect repair; but for patients with large skin defects, autologous skin sources are often insufficient to seal wounds in a timely and effective manner. Although allogeneic skin transplantation is a common clinical treatment for patients with extensive burns, the problem of immune rejection has been difficult to completely overcome, so it can only be used as a means to temporarily close the wound. In recent years, research and application of tissue engineering skin has made some progress, and it has provided new ideas and methods for solving this problem. However, there is still no ideal tissue engineering of skin that can fully meet the needs of patients with severe skin defects. One of the important reasons is the problem of the source of seed cells. Since the mature cells obtained from the patient's autologous or allogeneic cells are terminal cells and have no ability to further proliferate and differentiate to form new tissues, stem cells are the best choice for researchers in regenerative medicine [4].

So far, although there have been many breakthroughs in the application of stem cells and related technologies in skin wound repair and tissue regeneration, there are still many important problems that have not been proven, such as how to increase the proliferation of autologous cells, prolong the cells' life cycle and the ability to increase the secretion of cells; how to optimize the best cells of the same function from different tissue sources, establish a standard cell line, make the research work more comparable and scientific; the mutual interaction between cells and artificial extracellular matrix, the role and influencing factors, as well as the molecular mechanism guiding the development of pluripotent stem cells into target cells; how to accurately and efficiently transform the genome, and overcoming the immunological disorders during transplantation, will become the focus of long-term research.

## 9.3 Application of 3D Technology in Organ Tissue Engineering and Regeneration

With the development of regenerative medicine, the concept of 3D bioprinting (also known as organ printing) has received increasing attention. In 2010, 3D Bioprinters was named one of the 50 best inventions of 2010 by the Time magazine. Just like the normal 3D printing format, this biotechnology prints the tissue cells and the inner wall cells of the blood vessels layer by layer, which forms a solid organ that simulates human tissue. 3D printing technology was first proposed by Charles W. Hull in 1986. He called it "stereoscopic curing technology," which printed a thin layer of material after UV irradiation to form a solid 3D structure, which was then used to shape the biomaterial into a resin model with a three-dimensional structure. The discovery of solvent-free systems has enabled biomaterials to be printed directly to form 3D scaffolds for transplantation. 3D bioprinting as a tissue engineering technology and its further development requires the development of 3D printing technology, cell biology, and materials science. In clinical medical applications, 3D bioprinting has been used to make catheters and splints.

3D printing precisely controls the positioning of functional components such as biomaterials, biochemicals, and living cells in each layer, and prints layer by layer to form a 3D structure. Common 3D printing methods include biosimulation, autonomous self-assembly, and the construction of mini organization modules. At present, the development of new printing methods to create clinically active, biologically active 3D structures has attracted the attention of many researchers. One of the major challenges is transforming traditional printing plastic and metal technologies into new technologies that print sensitive, active biomaterials. However, the main challenge is to reproduce the microstructure of extracellular matrices and various cells with extremely high precision and to assess their biological functions (Fig. 9.1).

The main technologies used for biomaterial accumulation and molding are inkjet printing, micro-extrusion printing, and laser-assisted printing (Fig. 9.2). We discuss their differences from the most important factors of 3D bioprinting technology (interface resolution, cell activity, and biomaterials used for printing) (Table 9.1).

At first, 3D printing technology was only used in nonbiological fields, such as metal deposition, ceramics, thermoplastic polymers, and related technologies, such as organic solvents, high temperatures, and cross-linking reagents that are incompatible with biological materials and living cells. Therefore, finding materials that are compatible with both biomaterials and provide the appropriate mechanical and functional properties required for tissue scaffolding is a major challenge for 3D bioprinting.

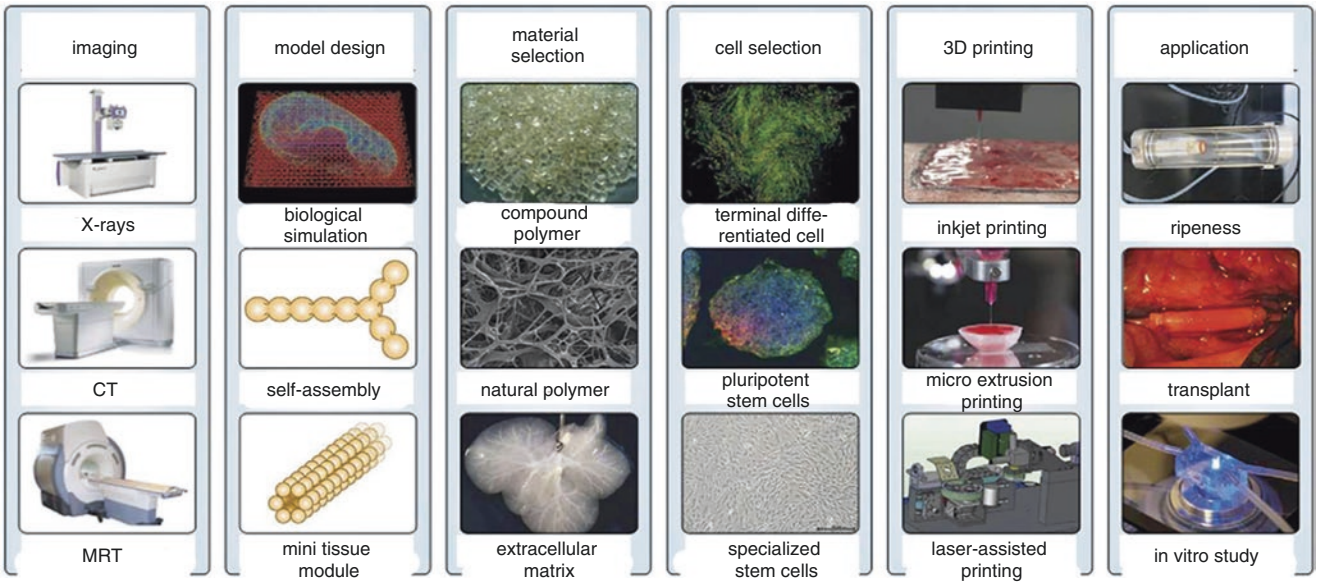


**Fig. 9.1** 3D bioprinting process

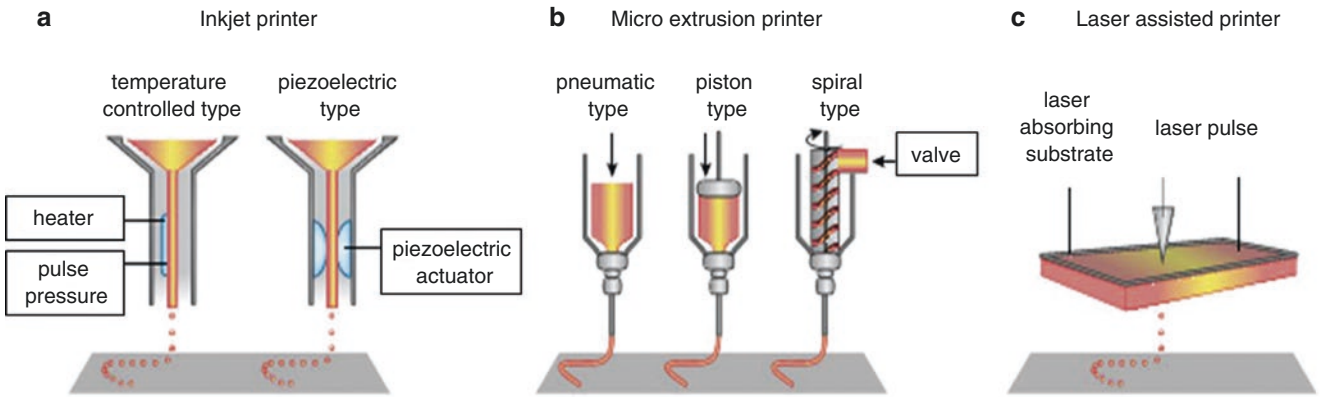


**Fig. 9.2** Composition and structure of an inkjet printer, micro-squeezing printer, and laser-assisted printer

**Table 9.1** Comparison of several bioprinters

| Bio printer type | | | | |
|---|---|---|---|---|
| | Ink Jet | Micro extrusion type | Laser assisted | References |
| Material viscosity | 3.5–12 mPa/s | 30–6 × $10^7$ mPa/s | 1–300 mPa/s | [5] |
| Molding method | Chemistry, optical cross-linking | Chemistry, temperature, optical cross-linking | Chemistry, optical cross-linking | [6] |
| Preparation time | low | Low to medium | Medium to high | [7] |
| Print speed | Quick (1–10,000 drip/s) | Slow(10–50 μm/s) | Medium to quick (200–1600 mm/s) | [8] |
| Print resolution | 50 μm | 5 μm | Microscale | [8] |
| Cell Survival | >85% | 40–80% | >95% | [9] |
| Cell density | Low, <$10^6$ cell/mL | High, cell mass | $10^8$ cell/mL | [9] |
| Print loss | Low | Medium | High | [10] |

Cellular 3D printing technology provides a new clinical medicine technology for tissue and organ repair, and also provides a very good research tool for research fields such as regenerative medicine, tissue engineering, stem cells, and cancer. In 2011, the Wake Foster Institute of Regenerative Medicine in the United States presented their human kidneys printed on a 3D printer in a TED (Technology Entertainment Design) presentation. They used the cultured kidney cells as a printed material and printed the cells layer by layer in a predesigned virtual model. The first layer is the cells and the second layer is the hydrogel used to bind the cells. Then repeat one layer until the entire kidney prints out. The initially formed kidneys are then moved to the incubator to provide nutrients for growth. By the time the cells survive, the hydrogel is degraded, leaving only the cells. At present, the laboratory said that it has been observed that this initial kidney model produced a urine-like substance, indicating that some kidney function has been obtained. Our laboratory uses the Regenovo bio 3D printer produced by a biotechnology company in Hangzhou to mix the biological hydrogel with the epidermal stem cells, construct a skin model in vitro, and successfully induce the epidermal stem cells to differentiate into skin accessories such as sweat glands, which brings hope to the perfect regeneration of the wound [11].

In 2013, Cornell University used the 3D printer to print the world's first artificial ear. Every year, tens of thousands of people lose all or part of their ears because of congenital development or illness or accident. Traditional ear replanting uses the costal cartilage as an alternative to ear cartilage, but the ear that is made out is neither aesthetically pleasing nor functional. The team used a computer to scan the normal ears of the patient's side, and then used a 3D printer to print a symmetrical ear model, in which the cell-filled collagen was injected as a scaffold for cartilage growth. The ears that are made out of this shape are suitable and beautiful in appearance. Within 3 months, these ears can grow cartilage and replace the collagen used for shaping. This research was published online in PLOS One magazine [12].

According to the May 2013 issue of the New England Journal of Medicine (NEJM), a 6-week-old boy in Ohio, USA, suffered from bronchial softening and was critically ill. The doctor used a 3D printer to create a splint that opened up a passage in the baby's airway. The baby boy finally managed to maintain his breath and survived. This is the first case of successful transplantation of 3D printed organs in medical history. According to the statistics of the US organ sharing network (UNOS), the number of patients waiting for organ transplantation is increasing year by year. However, due to the insufficient number of organ donations and the serious repulsion problems that may occur after surgery, traditional medical treatments have been unable to meet the requirements of organ transplant patients. Therefore, the application of 3D printing technology in this field will certainly bring about a medical revolution [13].

With the rapid development of 3D printing technology, the use of biocompatible materials, cells, and supporting components to print 3D functional organs is getting closer and closer to us. The future of 3D bioprinting technology will be a key technology for regenerative medicine to address tissue and organ transplant needs. Based on current trends, 3D printed tissue can be used not only for organ transplantation but also for drug delivery, analysis of chemical reagents, biological agents and toxic substances, and basic research. As we said before, 3D printing is very complex. Compared to nonbiological printing, 3D bioprinting involves many influencing factors such as material selection, cell type, cell growth, and differentiation factors, maximally maintaining cell viability and construction of tissue structure. There are many challenges that lie ahead of us, including the maturity and functionalization of the organization, the appropriate vascularization and neurochemistry. Starting from a 2D tissue such as the skin, a solid organ such as a kidney is finally formed by forming a hollow duct such as a blood vessel and a hollow tubular-free organ such as the bladder. Only by combining the knowledge of multidisciplinary fields such as engineering, biomaterials science, cell biology, physics, and medicine, can we solve these problems and realize the need for 3D bioprinting for regenerative medicine to solve tissue and organ transplants [14].

## 9.4 Tissue Engineering Cornea

### 9.4.1 The Classification of Tissue Engineering Cornea

Materials for constructing tissue engineering corneas mainly include scaffold materials and seed cells. There is no standard classification method for tissue engineering cornea in the world. At present, it is mainly classified according to the material of the scaffold and the source of the seed cells.

#### 9.4.1.1 Classification by Source of Scaffold Materials

According to the source of the scaffold material, the tissue cornea can be divided into synthetic materials and natural materials. The synthetic materials are subdivided into degradable polymer materials and nondegradable polymers. Natural materials are subdivided into natural polymer materials and biological derived materials.

A. Degradable Polymer Material
   Synthetic degradable polymer materials such as polylactic acid, polyglycolic acid, and polylactic acid glycolic

acid can be used as carriers for tissue engineering cornea [15]. These materials have good histocompatibility and support epithelial, stroma, and endothelial cell adhesion growth. These materials produce a large amount of lactic acid or glycolic acid during the degradation process in the body. Although they can be absorbed by the body gradually, and the metabolic mechanism is clear and biosafety is reliable, the acidic degradation products of these materials will adversely affect the activity of the cells. Therefore, it is not used as a scaffold material for tissue engineering corneal implantation, but it is tried in vitro to culture tissue engineering corneal seed cells.

B. Non-degradable Polymer

A cornea prepared using a non-degradable polymer is called an artificial cornea. Artificial corneas first appeared in clinical trials in the 1960s, initially using glass to sew directly onto the surface of the eye. Although this treatment can improve the patient's vision, due to the short-term rejection, the tissue around the artificial cornea dissolves and the artificial cornea falls off, which makes the surgery fail. With the continuous advancement of materials science, artificial corneas continue to develop, and the artificial corneas currently used in clinical practice are mainly BostonKPro [16] and AlphaCor [17], all of which have been approved by the FDA to enter the clinic.

The Boston I artificial cornea is the earliest applied artificial cornea. The optical cylindrical part is made of PMMA and has good transparency. The Boston II (Fig. 9.3) type is improved on the basis of type I, which optimizes the design of the artificial cornea, making the transplant surgery simpler, and the leakage rate of the suture is reduced. The titanium alloy fixture is added to reduce artificial corneal prolapse due to rejection (Fig. 9.4).

Osteo-Odonto-keratoprosthesis (OOKP) was pioneered by the Italian scientist Strampelli, using autologous tissue (teeth) as a peripheral scaffold and PMMA as an optical column. This special structure of the bone tooth is better for increasing corneal biocompatbility and binding to the host, which play a positive role. It has been reported that OOKP has been placed in patients for up to 20 years. Although the creative implantation of autologous tissue as part of the cornea reduces the rejection rate and visual acuity recovery after implantation, the complications are still unavoidable, and the operation is difficult and costly, which limits its wide application.

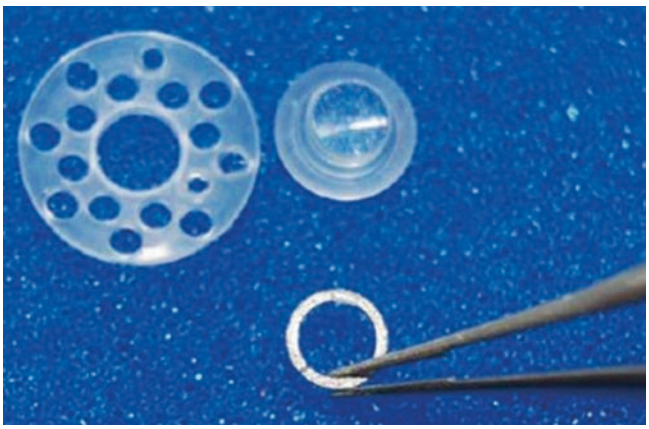

**Fig. 9.3** Boston II artificial cornea

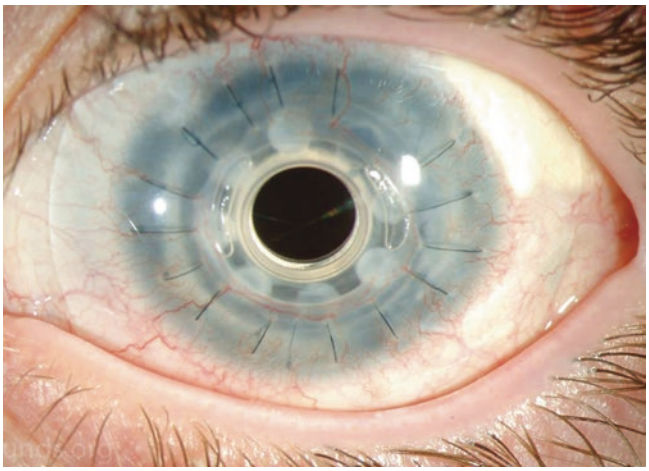

**Fig. 9.4** After Boston II artificial corneal transplantation

Due to the high rejection rate, the surgical indications for artificial corneas are relatively narrow, and patients with severe ocular surface trauma and severe damage to the conjunctival sac or tear system cannot be recovered. This is also one of the reasons why artificial cornea development is slower than tissue engineering cornea.

C. Natural Polymer Materials

Natural polymeric materials are derived from extracts of natural materials. In the early days, there were many studies on the use of natural polymer materials for tissue engineering cornea, and collagen was one of the commonly used materials. Collagen fiber is the main component of the corneal stroma, and collagen accounts for 75% of the dry weight of the cornea [18]. Collagen has no antigenicity, good histocompatibility, and contains certain specific amino acid sequences, which are beneficial to the adhesion and growth of seed cells. The shortcomings of collagen are poor stability, low mechanical strength, and rapid degradation (the degradation time of collagen produced by ordinary cross-linking method is about 1 month), and it is cross-linked by various physical or chemical methods, such as thermal cross-linking, UV and λ-ray cross-linking, and polyepoxide cross-linking, which can improve mechanical strength and resistance to degradation (the degradation time can be increased to 3 months or half a year).

Chitin is the only natural polysaccharide in the natural world that is second only to cellulose, and is widely found in insects, crustacean shells, and fungal cell walls [19]. The deacetylation reaction turns it into chitosan. Such natural polysaccharides have significant alkalinity, good biocompatibility, and biodegradability. Chitosan is hydrolyzed into oligosaccharides by the action of lysozyme and chitinase in the body. The degradation products are N-acetylglucosamine and glucosamine, which are nontoxic to the human body. The low-molecular-weight chitin or its oligosaccharide produced during the degradation process does not accumulate in the body and is not immunogenic.

Although there have been studies to continuously improve cross-linking and cross-linking methods to delay the degradation cycle of natural polymer materials, these materials cannot be transparent when used as a scaffold for corneal stroma, as a scaffold for corneal epithelium or corneal endothelium. Its biological properties are not as good as some biologically derived materials, such as amnion, so it is not widely used.

D. Biologically Derived Materials

Bioderived materials have certain advantages because their structures and biological components are closer to living tissues. The construction of tissue engineering corneas with amnion as a carrier has become a research hotspot. Amniotic membrane is a translucent membrane with no blood vessels, nerves and lymphatic vessels. It has low immunogenicity and is resistant to new blood vessels, inhibits fibrosis, and reduces scar formation. The amniotic basement membrane contains IV-type and V-type collagen, laminin, fibronectin, and various chelating proteins, which are beneficial for cell differentiation and migration. At present, amniotic membrane has been widely used in tissue engineering research, and the corneal epithelial layer, stromal layer, and endothelial layer have been successfully constructed. Amniotic membrane is considered an ideal carrier material for tissue engineering cornea [20]. However, the amniotic membrane is too thin to construct a stratified corneal tissue. The application of the amniotic membrane also has the risk of causing the spread of infectious diseases such as hepatitis and acquired immunodeficiency syndrome (AIDS).

Other tissue-derived thin carriers can be used to culture epithelial and endothelial seed cells to construct tissue engineering corneas, primarily postcorneal elastic layers, amnion basement membranes, synthetic gelatin membranes, anterior capsular membranes, and skin-derived fibrous membranes.

#### 9.4.1.2 Classification by Seed Cell Source

According to the source of seed cells, it can be divided into adult stem cells, embryonic stem cells, and induced pluripotent stem cells (iPSC). The main sources of adult stem cells are corneal epithelial stem cells and bone marrow mesenchymal stem cells. The former is mainly obtained through corneal donation and autologous biopsy, and the latter source can be obtained by sorting out bone marrow mesenchymal stem cells in the blood of the patient. Embryonic stem cells come from a wide range of sources and are mainly obtained by cord blood. The source of autologous stem cells is limited, and allogeneic stem cells have a certain rejection rate, and iPSC will be able to solve these factors that limit the development of tissue engineering cornea.

### 9.4.2 The Construction Method of Tissue Engineering Cornea

The construction of tissue engineering cornea refers to the combination of seed cells and scaffold materials to form a firm, unitary structure and functional unit by culture in vitro. The construction methods of tissue engineering corneal epithelium, stroma, and endothelium are described below.

#### 9.4.2.1 Construction Method of Tissue Engineering Corneal Epithelium

Sources of corneal epithelial seed cells: in the normal basal layer of the corneal epithelium, there are stem cells and transiently expanded cells. The corneal epithelial stem cells are small in volume, large in nucleoplasmic ratio, and positive for CK19 and CK15, suggesting that the cell proliferation ability is strong [21]. It is generally believed that corneal epithelial stem cells do not express corneal epithelial-specific surface markers CK3 and CK12, and positive markers include Importin13, p63, ABCG-2, integrin α9, and N-cadaherin. Due to the limited source of corneal epithelial stem cells, it has been found that some adult stem cells from other sources can be transformed into epithelial cells with functional properties of corneal epithelial cells by in vitro expansion and induced differentiation. Sources of tissue that have been discovered so far include bone marrow, oral mucosa, fat, skin, and circulatory systems (Fig. 9.5).

In recent years, the development and improvement of limbal stem cell culture technology has provided a platform for transplantation of cultured limbal stem cells [22]. The source of the cells may be the limbus itself or the limbus of the allograft. The carriers available for transplantation include amniotic membrane, eggshell membrane, polylactic acid membrane, contact lens, cellulose membrane, etc., in which the epithelial cell amniotic membrane has more organisms. It is suitable for the growth of stem cells and has the effect of improving the inflammatory environment of the ocular surface. Therefore, it is an ideal carrier for transplantation and lays a foundation for clinical transplantation of cultured cells. At present, the technique of in vitro-cultured corneal

**Fig. 9.5** Amniotic membrane cocultured with stem cells

epithelial stem cell transplantation for reconstructing the ocular surface has matured, but how to shorten the culture time and maintain the characteristics of the limbal stem cells after culture still need further study.

#### 9.4.2.2 Allogeneic Corneal Decellularization Method

The allogeneic-derived cornea can be an extracellular scaffold for tissue engineering cornea by decellularization [23]. There are currently more than ten methods in the world for preparing a lamellar tissue engineering cornea by removing animal-derived corneal stromal cells. It mainly includes the following three types: a. Chemical reagent method: It destroys proteins, lipids and DNA in cells by using an acidic solution, an alkaline solution, a detergent, etc., and dissolves cell debris in a solvent to separate from the extracellular matrix. b. Biological reagents: Cells are inactivated and removed from the scaffold material by hydrolysis of catalytic nucleotides, deoxynucleotides, or peptide chains using nucleases and proteases. c. Physical decellularization method: By changing the physical temperature, pressure, osmotic pressure, and other physical factors outside the material, it will destroy the cell membrane and nuclear membrane, but cannot very effectively inactivate the cells and remove from the scaffold material.

#### 9.4.2.3 The Construction Method of Tissue Engineering Corneal Stroma

The source of corneal stromal seed cell: Some scholars believe that there are stem cells in the corneal stroma that use collagenase to decompose the corneal stroma, obtain corneal stromal cells, and select corneal stromal cells by cloning and culture. These expression stem cells have specific surface markers, such as Bmi-1, Notch-1, Six2, Pax6, ABCG2, Spag10, p62, and the like. Bone marrow mesenchymal stem cells can be used as one of the sources of corneal stroma seed cells.

#### 9.4.2.4 Construction Method of Tissue Engineering Corneal Endothelium

Sources of corneal endothelial seed cells: Human corneal endothelial cells in vivo cannot be propagated by mitosis, which is due to insufficient growth factor in the anterior chamber, and growth inhibitory factors in the aqueous humor, which block the DNA synthesis of the cells. However, the presence of various growth factor receptors on the corneal endothelium indicates that endothelial cells have potential for division. The growth and proliferation of corneal endothelial cells in vitro is also affected by cell origin. It has been shown that corneal endothelial cells in infants can be mitotic under certain conditions; the success rate of endothelial cell culture in patients over 20 years old is greatly reduced. Therefore, in the experiment of human corneal endothelial cell transplantation, the cultured endothelial cells are mostly from fetuses, infants, or young people under 20 years old [24].

Normal adult corneal endothelium does not proliferate under physiological conditions, but some endothelial cells express stem cell surface markers after trauma. McGowan believes that stem cells of corneal endothelial cells exist in the transitional region between trabecular meshwork and endothelial cells. In animal experiments, central and peripheral corneal endothelial cells have a certain proliferative capacity.

Construction of tissue engineering corneal endothelium: The method of tissue engineering corneal endothelium is to use a vector to grow into a continuous sheet of monolayer cell membrane for transplantation in vitro.

#### 9.4.2.5 Construction Method of Total Corneal Tissue Engineering

Tissue engineering of the whole cornea is constructed by cocultivating the corneal epithelium and its carrier, the corneal stroma scaffold and its seed cells, and the monolayer of endothelial cells according to the histological morphology of the normal cornea.

3D printing technology is currently being tested in the full corneal construction technology. Currently available printing materials are natural polymer materials such as agar, gelatin, collagen, and cellulose. Therefore, when the tissue engineering cornea after surgery is reconstructed, it cannot be like the corneal reconstruction after human corneal transplantation, and the transparency of the graft was decreased due to the growth of receptor cells and extracellular components into the tissue engineering cornea.

### 9.4.3 The Clinical Application of Tissue Engineering Cornea

Tissue engineering corneal epithelium progresses fastest in the direction of tissue engineering cornea. Italy, India, Japan, China, and other countries have used it in clinical practice for nearly ten years, and they initially evaluated the therapeutic effects of different culture methods and different surgical methods on tissue engineering corneal epithelial stem cell transplantation.

The clinical research of tissue engineering corneal stroma in China is at the forefront of the world. The large-scale clinical trials of tissue engineering corneal stroma in China have proved its safety and effectiveness. China's tissue engineering corneal stroma has entered the stage of industrialization, which will lay a good foundation for the development of clinical tissue engineering cornea in China.

### 9.4.4 Development Direction and Challenges of Tissue Engineering Cornea

#### 9.4.4.1 Development Direction of Tissue Engineering Cornea

Tissue engineering corneal epithelial technology has matured. With the establishment of industrialization standards such as culture, storage, and transportation of corneal epithelial stem cells, tissue engineering corneal epithelium will be widely used. At present, the most successful research on the synthesis of natural materials is the method of cross-linking type I collagen and type III collagen by aldehydes in Griffth, Canada (Fig. 9.6), and constructing a transparent corneal stroma stent with a thickness of 400 μm and transplanting it to 20 inpatients with corneal leukoplakia and keratoconus; the corneal epithelium was observed intact and the matrix was transparent 24 months after surgery [25]. The results of this research point to a new direction for the construction of tissue engineering corneal stroma for natural materials.

The tissue engineering cornea of allogeneic origin has many advantages such as wide source, low price, and simple preparation method. In animal experiments and early clinical trials, it was observed that the porcine-derived lamellar tissue engineering cornea can remain transparent for a long time. In vivo confocal microscopy revealed that 1 year after tissue engineering corneal transplantation, the subcutaneous plexus of cornea and the corneal stromal cells and nerve fibers in the stroma grow into the tissue engineering cornea. The results of this study demonstrate that pig-derived lamellar tissue engineering cornea is suitable for use as an allogeneic tissue engineering corneal scaffold with good clinical application prospects (Fig. 9.7).

The rejection rate after allogeneic corneal endothelial transplantation is higher than that of other types of corneal transplantation, so the requirements for tissue engineering corneal endothelium seed cells are higher than other seed cells. Due to the limited source of endothelial cells, the development of tissue engineering corneal endothelium technology is currently limited.

#### 9.4.4.2 The Challenge of Tissue Engineering Cornea

The ideal scaffold material has always been a bottleneck restricting the development of tissue engineering cornea. At present, although a wide range of materials have been extensively studied, there are not many scaffold materials that really meet the needs of constructing tissue engineering

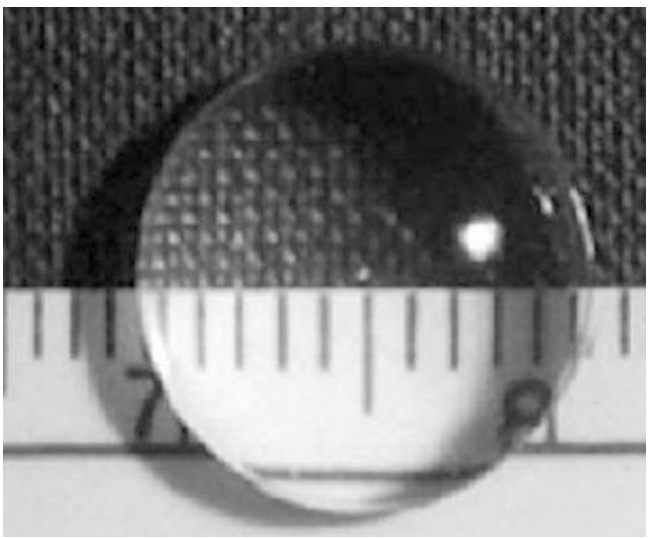

**Fig. 9.6** Tissue engineering cornea cross-linked with collagen

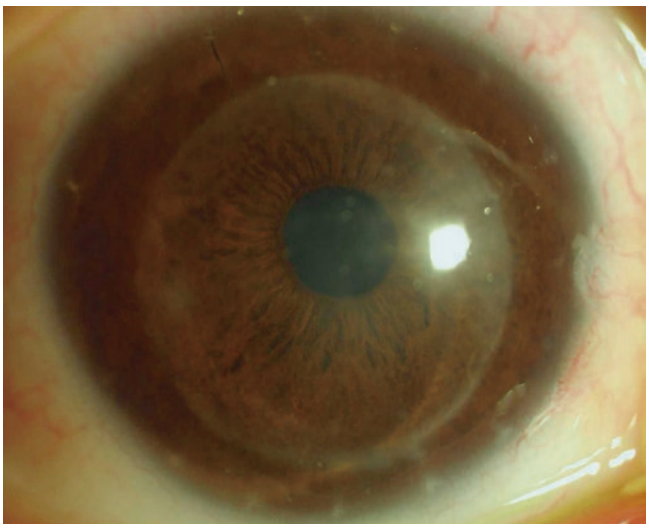

**Fig. 9.7** 3 Years after porcine-derived tissue engineering corneal transplantation

corneas, and both have certain disadvantages. Natural polymer materials, such as collagen, hyaluronic acid, chitin and its derivatives, and polysaccharide polymer materials, have good biocompatibility, but have disadvantages such as poor mechanical properties, too fast degradation, and are not conducive to the diffusion of the cellular nutrients into the material. Acidic degradation products of synthetic polymer materials tend to accumulate locally, which is not conducive to cell growth. Bioderived materials are derived from natural tissues and have certain development prospects as tissue engineering scaffold materials. However, its safety, tissue transparency, stability, and mechanicality need to be further evaluated before entering the scale of clinical trials.

In addition, the challenges faced by tissue engineering corneas include ethical restrictions, lack of relevant laws and regulations, and the lack of standardized source standards, preparation and preservation methods, and clear clinical indications.

Compared with other tissue engineering tissues and organs, tissue engineering corneas in China have developed rapidly. These clinical research advances indicate the advent of tissue engineering corneal industrialization, and the subsequent clinical research and the application will provide more choices and help for humans to cure corneal diseases.

## 9.5 Tissue Engineering Tendon

Tendon and ligament damage caused by trauma, exercise, chronic strain, and other injuries is a common clinical problem, which can lead to dysfunction and even disability. Since the advent of modern surgery, the most commonly used methods for repair of tendon are autograft, allograft, or artificial material, but they are still facing many problems.

As far as we know, the ideal tendon substitute should have the following advantages: (1) good histocompatibility and biomechanical properties; (2) does not adhere to the surrounding tissue after implantation; (3) can secure suture knots and smooth junction of tendon ends; (4) the healing speed is at or near the normal physiological healing process; (5) the tensile strength can match to the needs of the body; (6) easy to obtain or manufacture; and (7) can be assimilated by the body into a part of the body. Overall, the most important aspects of tendon substitute materials biology are able to completely replace the function of the tendon, and accept the regulation of the body and participate in the self-renewal of the body. The aim of tendon tissue engineering research is to develop a tendon substitute that meets the above requirements as much as possible. The principle is to obtain the seed cells of the tendon, and then combine with the biomaterial to form a complex after culture and amplification, and implant it into the defect site of the tendon to implant. The seed cells continue to proliferate, differentiate, and secrete the extracellular matrix to form repaired tissue, and then the biological material gradually degrades, eventually achieving complete repair in a biological sense.

In the following, we will begin with the structure and nutrition of the tendon, biomechanics, damage patterns, etc., and outline the damage and repair of tendon, the research on key technologies such as tissue engineering tendon seed cells, the study of scaffold materials, the effect of cell growth factor on tendon healing, composite culture of tendon cells and scaffold materials, as well as the practice in preclinical and clinical transformation applications, share the history of tissue engineering tendon research and development, in order to better promote the transformation of tissue engineering tendons.

### 9.5.1 The Damage and Repair of Tendon

#### 9.5.1.1 Structure and Nutrition of Tendon

The tendon is a dense connective tissue that connects the skeletal muscle to the bone [26]. Each muscle has different lengths of tendon and bone attachment. Because of the contraction of muscle belly, the tendon is pulled to drive the bone to produce movement, so that the human body can complete various activities of life and the various movements required for work. Thus tendon play a very important role in the life activities of people. It is not only the main support structure of the human body but also an important organization for accomplishing various activities, production labor, and participating in social activities.

A. Structure of Tendon

The tendon is composed of extracellular matrix and tendon cells [27]. Tendon cells belong to the class of fibroblasts, accounting for about 20%. The tendon cells are fusiform in the living body, arranged in a row, and projecting the wing-like protrusions around the collagen fibers. The extracellular matrix such as collagen, elastin, and glycoprotein secreted by tendon cells accounts for 80%, of which collagen accounts for 65–70% of the formed fraction, mainly type I and a small amount of type III collagen, but there are many other types of collagen, such as IV, V, and VI. The collagen fibers are white in color, varying in thickness, with diameters ranging from 1 to 12 μm [28]. There are alternating periodic stripes of light and dark, and the horizontal cycle is about 6.4 μm, which is a unique feature of collagen fibers. The collagen fibers have branches, and the branches are interwoven into a net to allow the collagen fibers to withstand large tensile stresses. Collagen fibers are cross-linked, making the mechanical properties of collagen more stable and the tensile strength of tissue higher. Collagen cross-linking is mainly carried out by disulfide bonds

formed between cysteines. The tendon also contains a certain amount of elastin, which gives the tendon and ligament tissue a certain elasticity.

Proteoglycans are another important component of the extracellular matrix of tendons and play an important role in the formation of viscoelastic and other mechanical properties of tissues [29]. Proteoglycans are macromolecules composed of glycosaminoglycan chains covalently bound to a protein core. These branched chains with negative electrical properties form a characteristic "bottle brush" structure that attracts water molecules to hydrate tissue, while proteoglycans are embedded between collagens to provide compressive properties of tissue, hydration also enable the water-soluble molecules to rapidly disperse in the tissue.

B. Nutrition and Metabolism of Tendons

Human tendon is the kind of tissue with low blood supply and low metabolism and its nutrition is supplied by a diffusion process from the s nutrient vessels and synovial tissue [30]. In addition, metabolic activity is regulated by synovial membrane and blood vessels. Recent studies on the distribution of tendon blood vessels suggest that although tendons are less blood donor tissues, they still have an intrinsic vascular distribution, which provides nutrients and material exchange for tendons. Finally, it should be noted that further studies should pay attention to the vascularization process of engineered tendon in the research of tendon tissue engineering.

The tendons in the sheath region of the human body lies within the synovial fluid system [31]. Synovial fluid is a transparent, slightly yellow viscous liquid which is formed by the exudation of protein-rich fluid from synovial capillaries and by the addition of proteins and protein polysaccharides synthesized by the synovial lining cells. The major constituent of the proteins is mainly hyaluronic acid, the content of which is about 2 mg/mL in the normal sphincter synovial fluid. It is not only the main source of nutrients for intrathecal tendon but also provides a lubricant for the sliding of tendon. The synovial structure on the surface of the tendon plays an important role in the metabolic activities of tendons. Observation of the tendon ultrastructural found that the synovial membrane had micro pores and a network structure between the endometrium and the iliac membrane, allowing the synovial fluid to pass freely. When the muscles relax, the tendons absorb the synovial fluid like a sponge into the tendons and nourish the tendons; when the muscles contract, the internal pressure of the tendons rises, the synovial fluid is squeezed into the sheath, and the metabolites are discharged. It is suggested that the microporous structure required by this synovial nutrient should be taken into account when designing scaffold materials for tissue-engineered tendons.

#### 9.5.1.2 Biomechanics of Tendon

The structural characteristics of the tendon endow it strong tensile strength and a certain viscoelasticity [32]. The movement of the body can also cause the reconstruction of the tendon tissue structure and change its biomechanical properties. Several studies have found that proper mechanical stimulation of the tendon alters the production of collagen type I, and the synthesis of collagen I is increased when the anabolism synthesis is increased. The stress stimulation results in a series of changes in gene expression, which further lead to structural changes in tendons. Periodic stretch stress can stimulate the content of various growth factors, such as TGF-β, PDGF, bFGF, thereby promoting the proliferation, differentiation, and matrix synthesis of tendon cells, the secretion of IL-6 is also increased, and IL-6 is involved in the inflammatory response and thereby promotes tendon repair.

In growth, development, and various physiological and pathological processes, due to the change of stress, the synthesis of collagen fibers undergoes adaptive changes, including the reconstruction of various types of collagen content and spatial arrangement. Age is another important factor influencing the mechanical strength of tendons. As the age increases, the intramolecular and intermolecular cross-links of collagen increase, which makes the collagen fibers hard and brittle, and changes the mechanical properties of the tendon.

#### 9.5.1.3 Injury and Healing of Tendon

Acute and chronic injuries are the two main modes of injury to the tendon, and it varies depending on the treatment method. Tendon healing follows the classical wound closure process that is comprised of three conjunct steps, namely inflammation, formation, and remodeling [33].

The source of healing cells, the source of nutrients, and the voids present in the muscle-tendon complex rupture and conservative treatment within the tendon healing process will have an important impact on tendon healing [34]. The first is the way of the tendon healing. Endogenous healing is completely dependent on the cells of the tendon tissue itself. However, exogenous repair relies on cell infiltration of external tissues (including the tendon sheath). Normally, these two mechanisms normally act cooperatively in the repair process of the tendon: exogenous repair mainly functions in an early stage, while endogenous repair functions in the late stage. The result of exogenous healing is scar formation and it forms adhesions to the surrounding tissues in the healing process of flexor tendons, which may impede normal range of motion. Second, it is the source of nutrition. Blood vessels supply oxygen and nutrition to the most areas during the tendon healing process. While at the trochlear part, tendon nutrition is primarily dependent on the penetration of nutrients in the synovial fluid. Next, a gap between the broken ends will directly affect the range of motion of the tendon,

but also the mechanical properties of the healing tendon. Finally, it is the treatment modality of choice. Generally, whether conservative treatment and/or surgical treatment are effective mainly depends on their respective indications. In comparison, surgical treatment can better promote the healing between the broken ends of the tendon; especially, the repair of the flexor tendon of the hand often requires surgery to promote a good fit between the broken ends of the two tendons and subsequent good healing and functional rehabilitation.

#### 9.5.1.4 Repair of Tendon

The most basic method of repairing tendon is direct suture and tendon transposition. If there is a tendon defect, it will require bridging between the broken ends. Bridging materials can be derived from autologous, allogeneic, xenogeneic, artificial materials, and tissue engineering materials. Among these materials, autogenous materials are the most commonly used conventional methods. The implantation autologous materials used to repair tendon defects are largely classified into autologous tendons and autologous fascia strips. Allogeneic tendon transplantation for repairing tendon defects avoids the limitation of autologous transplantation regarding materials, but the obtained materials need to special treatment, otherwise the repair effect will be reduced. Xenogeneic tendon and artificial materials to repair tendon defects are still in the experimental stage. However, some noteworthy progress has been made.

As far as we know, the ideal tendon substitute should have the following advantages: (1) good histocompatibility and biomechanical properties; (2) does not adhere to the surrounding tissue after implantation; (3) can secure suture knots and smooth junction of tendon ends; (4) the healing speed is at or near the normal physiological healing process; (5) the tensile strength can match the needs of the body; (6) easy to obtain or manufacture; and (7) can be assimilated by the body into a part of the body. Overall, the most important aspects of tendon substitute materials biology are able to completely replace the function of the tendon, and accept the regulation of the body and participate in the self-renewal of the body. The aim of tendon tissue engineering research is to develop a tendon substitute that meets the above requirements as much as possible. The principle is to obtain the seed cells of the tendon, and then combine with the biomaterial to form a complex after culture and amplification, and implant it into the defect site of the tendon to implant. The seed cells continue to proliferate, differentiate, and secrete the extracellular matrix to form repaired tissue, and then the biological material gradually degrades, eventually achieving complete repair in a biological sense.

The concept and research progress of tissue engineering tendon provides a new, more ideal treatment method for tendon injury consistent with physiological characteristics. The main contents of tissue engineering tendon include seed cells, scaffold materials, growth factors, cell and scaffold material composite culture.

### 9.5.2 The Seed Cell Research of Tissue Engineering Tendon

The tendon tissue includes the aponeurosis, the tendinous fibers, the blood vessels, and lymphatic vessels between the tendons [35]. The cell components also include fibroblasts, synovial cells, vascular endothelial cells, and tendon cells. The functional cells of the tendon tissue are tendon cells. At present, there are mainly some types of the seed cells used to study, such as tendon cells, skin fibroblasts, and stem cells, and they have their pros and cons.

#### 9.5.2.1 Tendon Cells

Tendon cells are intrinsic cells of the tendon [36]. The tendon cells, in morphological classification, belong to the class of fibroblasts. Tendon cells are highly differentiated cells. Tendon cell proliferation is relatively slow in in vitro culture conditions, and the tendon cells even lose their proliferative abilities after multiple passages. Therefore, this is unfavorable for the study of tissue engineered tendons.

Xie Huiqi et al. systematically studied the biological characteristics of human embryonic tendon cells, and found that the morphology of tendon cells and the function of secreting type I collagen were changed after 13 passages [37]. Besides, the replicative senescence phenomenon appeared. To resolve this issue, transfected human tendon cells with ptsA58H plasmid and the cell proliferation ability were enhanced after transfection. Furthermore, it can be passed on serial passage, long-term storage, and long-term recovery without alterations in growth characteristics. Thus a standard cell line was established for tissue engineering research with no tumorization tendency and relative immortalization. This cell line was used to filtrate and evaluate various tissue engineering scaffold materials of tendon.

#### 9.5.2.2 Fibroblasts

The source of fibroblasts is widely distributed compared with tendon cells, and it is easy to obtain [38]. It has the same biological characteristics as the mesoderm-derived cells of the tendon cells. They are all fusiform and can be synthesized and secrete collagen, elastin, glycosaminoglycan, and glycoprotein. Experiments have confirmed that skin fibroblasts can survive well on the surface of scaffold materials. By comparing the tissue engineered tendons constructed by skin fibroblasts and tendon cells, Chen Bing et al. observed that the gross morphology, histology, collagen orientation, and biomechanical properties were similar. After 26 weeks,

the cell matrix ratio of the experimental group reached a satisfactory level and the mechanical strength reached 74% of normal tendon. It is suggested that fibroblasts can be used in place of tendon cells for tendon tissue engineering.

#### 9.5.2.3 Stem Cells

A. Bone Marrow Mesenchymal Stem Cells

Bone marrow mesenchymal stem cells (BMSCs) can be easily obtained, have multi-directional differentiation potential after in vitro culture and cryopreservation, stable genetic background, no immunological rejection in vivo, and are easy to use for clinical application [39]. Therefore, BMSCs have become an ideal seed cells in tissue engineering. Research shows that BMSCs have the ability to secrete type I collagen without induction in vitro, which is the same as the main function of tendon cells.

Young et al. seeded the proliferating BMSCs on the collagen gel and implanted the complex on the tendon defects [40]. It was found that the tendons treated with BMSCs were thicker than the control group, and alignment of collagen fibers, the quality of the joints, and biomechanical properties are better than that of the control group. Long Jianhong et al. inoculated isolated BMSCs onto type I collagen polyglycolic acid scaffolds. BMSCs grew well after 2 weeks of mixed culture, maintaining more than 89% cell viability. Transmission electron microscopy results showed that the cells in the experimental group still maintained a strong secretion function. It indicates that the collagen-polyglycolic acid has good cell compatibility with BMSCs.

Studies have found that BMSCs can lead to ectopic osteogenesis in tendon repair, suggesting that effective regulation of BMSCs specific differentiation into tendon cells is an important prerequisite for stem cell application. In addition, the cell source of BMSCs is also an important problem. Currently, there is still no perfect solution for obtaining and cultivating BMSCs to obtain a sufficient number of functionalized tendon-like cells, and the efficiency of directed differentiation is not ideal, and the related research needs to be further carried out.

B. Tendon Stem Cells

In 2007, Bi et al. successfully isolated tendon-derived stem cells (TDSCs) from mouse and human tendon tissues [41]. Later, other researchers isolated TDSCs from tendon tissues of horses, rabbits, and rats. It has been identified that these TDSCs derived from different species have the following characteristics: expression of stem cell-related markers; have the capacity to form clones; and self-renewal ability. Under suitable conditions or appropriate induction, MSCs can differentiate into a variety of tissue cells, such as osteogenesis, adipogenesis, and chondrogenesis in vitro, and muscle-like, cartilage-like, bone-like, and tendon-bone-like tissues are formed after subcutaneous implantation in nude mice.

Extensive clinical data have demonstrated that ectopic cartilage and ossification are common in the tendon healing process or tendinitis. This may be due in part to the multidirectional differentiation potential of TDSCs. However, it is because these TDSCs have these characteristics of stem cells that we can apply TDSCs as seed cells to tendon tissue engineering. PP Lui and his team found that culturing TDSCs into the lamellar structure and then wrapping them on the graft surface can help repair the anterior cruciate ligament in rats, and the TDSCs transfected with Scleraxis can better promote the repair of patellar tendon defects in rats [42]. At the same time, TDSCs can also be used in combination with other bioderived substrates, such as platelet-rich plasma (PRP), which can promote the proliferation and differentiation of TDSCs by using abundant growth factors in PRP. Lei C et al. compared the pure PRP, TDSCs, and TDSCs-PRP complexes in the treatment of rat Achilles tendinitis, and found that the repair ability of TDSCs-PRP complex was significantly stronger than that of pure PRP and TDSCs.

Currently, although TDSCs are the ideal seed cells for tendon tissue engineering, there are still many problems and deficiencies in their research. First, purification and expansion of a cell population containing only TDSCs are still difficult, because the TDSCs we have isolated also contain a part of tendon cells. Next, the research on the microenvironment of TDSCs in vivo is not thorough enough, and it is impossible to completely construct each factor involved in the microenvironment. Again, the problem of the source or quantity of TDSCs and how to meet the needs of scale clinical application of tendon repair remains to be solved. Therefore, using TSPCs as the seed cells for tendon-bone tissue engineering might need researchers' further in-depth exploration and research.

C. Other Stem Cells

It has been reported in the literature that adipose-derived stem cells (ASCs) can be used for the treatment of tendons. Whether ASCs have similar inducibility and ability to differentiate into mature tendon cells like BMSCs is also a scientific problem worthy of discussion.

Embryonic stem cells (ESCs) are also an important source of cells for tissue regeneration. Chen et al. were the first to propose the mesenchymal stem cells isolated from mouse embryonic stem cells. Cell sheets of ESCs derived MSCs were engineered into tendon-like layers under static mechanical load in vitro and used to repair a window defect in the patellar [43]. The results showed that mechanical stimulation can induce these cells into

tendon-like cells, to form tendon-like structures, and to express tendon cell-associated markers. In addition, these cells can promote the regeneration of defect tendons after implantation in vivo. The results suggest that embryonic stem cells have multi-directional differentiation potential, which may be a good cell model for studying tendon cell development and stem cell-induced tendon differentiation. However, in practice, several issues need to be addressed, such as ethics, immune rejection, and biosafety.

### 9.5.3 Study on Scaffold Material of Tissue Engineering Tendon

Due to the unique structure and strong mechanical properties of the tendons, the ideal scaffold should cover a number of requirements such as: (1) It has good biodegradability, and its degradation rate should match the tissue regeneration rate; (2) The material itself or its degradation products have good biocompatibility to the cells and the tissues of the host, whether before, during, or after degradation of the material; (3) It has excellent mechanical properties and still maintains good mechanical properties during tissue regeneration; (4) It has good biological properties, which are beneficial for cell attachment, proliferation, differentiation, matrix secretion, and tissue formation; (5) It has excellent machinability, which can be easily prepared in different special structures and shapes, such as can be further knitted or woven.

#### 9.5.3.1 Scaffold Material

A. Natural Polymer Material

Silk, chitosan, and collagen are the most widely used natural polymeric materials in the biomaterials, which possess superior properties such as good tissue compatibility and can retain the normal structure of the network. However, there are still some shortcomings such as unadjusted degradation rate, residual material after disintegration, body rejection, difficult to process, and shape. Thus, more investigations are required to further research.

B. Synthetic Materials

When used as scaffolds for tendon tissue engineering, the advantages of synthetic materials are as follows: easy to obtain, stable in physical and chemical properties, nontoxic, and nonantigenicity. Therefore, it has long been used in the research of tendon tissue engineering, such as polyester, nylon, and silicone rubber.

At present, commonly used artificial polymer materials in tissue engineering are polyglycolic acid (PGA), polylactic acid (PLA), polylactic acid-polyglycolic acid copolymer (PLGA), polycaprolactone (PCL), polyvinyl alcohol (PVA), and so on. Bin et al. cultured the tendon cells in combination with PGA fibers for 6 weeks and implanted them in nude mice. It was found that cell-containing PGA fibers could become a tendon substitute [44]. Qin Tingwu et al. combined the PGA with tendon cells and implanted them into the deep flexor defect of the chicken [45]. It was found that the PGA degradation was too fast, and the biomechanical properties of the new tendon were lower than that of the normal tendon. In the study of PVA as a tendon scaffold material, it was found that PVA has good histocompatibility and mechanical properties, but pure PVA has poor cell adhesion. The PLGA was made into a film and the surface of the membrane was lined with different substances. The tendon cells were inoculated on the PLGA membrane and the adhesion of the tendon cells to the polymer film was measured. It was found that type I collagen and fibronectin mediate the specific adhesion of the tendon cells to the membrane, and this interaction can be inhibited by the corresponding antibody molecule. In addition, the synergistic effect can be produced when the concentration of the composite lining reaches a certain ratio. Moreover, the growth factor has an obvious effect in promoting tendon cells adhesion.

As a scaffold material, synthetic polymer materials have the advantages of abundant sources, easy processing, adjustable degradation rate, but their mechanical strength is low, the hydrophilicity and adhesion ability are poor, and the tissue compatibility is not ideal. However, synthetic polymer materials are still in need of further investigation and improvement, including surface modification, improving the biocompatibility of materials, reducing the body's immune response to biological materials, and reducing the impact on tendon healing.

C. Composite Materials

Due to the obvious defects of natural materials and synthetic materials, their use alone may be limited. Therefore, the combination of the two may be beneficial for improving the overall performance of the material and meeting the application requirements. The repair effect was better in the silk fibroin-collagen group than collagen alone for rabbit tendon defect. The composite material had good mechanical properties and could promote the formation of collagen fibers. Thomas et al. also found that silk fibroin/PLGA composites have good cytocompatibility, which could promote cell proliferation and collagen production, and have a certain potential in clinical application [45]. Long Jianhong et al. inoculated MSCs into the type I collagen-polyglycolic acid scaffold to observe cell growth, and found that there was no significant change in cell functional activity. The results indicated that collagen-polyglycolic acid is a good biodegradable material for carrying MSCs. The

research team led by Yang Zhiming used a variety of composite materials, such as carbon fiber combined with PGA, treated human hair combined with PGA, collagen combined with PGA, and human hair and collagen combined with PGA. It found that the collagen secreted by tendon cells was distributed along scaffold material and gradually replaced the degradable part during the PGA degradation process. In addition, the mechanical properties of the newly formed tendon and the adhesion of the tendon cells were significantly improved. After 3 months of implantation, the tensile strength of the new tendon reached 75% of that of the original tendon, and the amount of collagen secreted by tendon cells was increased.

D. Biologically Derived Materials

Bioderived materials are also a large class of natural scaffold materials, which have gradually become the focus of tendon tissue engineering research. Compared with synthetic materials, bioderived materials possess the advantages of sufficient sources, closest to the native network structure, good biomechanical properties, good biocompatibility, and so on. Typical bioderived tendon materials are derived from animal or allogeneic tendon, which are treated by a series of chemical methods to remove cellular components and retain collagen scaffolds. The obtained materials still retain all the bioactive phytochemicals and enzyme activity after rehydration. These natural scaffolds are involved in tissue development, and these molecules then interact with cell surface receptors. This signal is then transmitted across the membrane to the intracellular molecules which trigger a serious of cascade reactions from the cytoskeleton to the nucleus, and thereby lead to specific gene expression. In turn, these specific gene products affect the extracellular matrix in a number of ways. The interaction of cells with the extracellular matrix promotes cell adhesion, migration, growth, and death, as well as the regulation of cytokines and growth factors, while indirectly activating the transmission of signals between cells.

So far, it is not certain which scaffold material is the best material for tendon tissue engineering research. It involves multidisciplinary fields such as material science, chemistry, engineering, bioengineering, cytology, and medicine. It requires a close combination of disciplines, joint research, and continuous exploration to find a more ideal practical tendon tissue engineering scaffold material.

#### 9.5.3.2 Preparation Technology of Scaffold

The artificial tendon fabrication technique not only requires the prepared scaffold that can promote cell proliferation and migration but also has sufficient overall structural properties to maintain a certain shape in vivo. There are two main methods for preparing tendon tissue engineering scaffold materials.

A. Electrospinning

The scaffold material obtained by electrospinning can mimic the natural extracellular matrix at the nanometer scale, which would have the characteristics of fine size, large specific surface area, high porosity, large aspect ratio, and superior mechanical properties. Bioscaffold materials with different properties can be obtained by blending different substances. At present, the electrospinning process has made great progress in the fields of cartilage and tendon regeneration. Lucy et al. used electrospinning technology to prepare PCL nanofibers for the repair of tendon injuries. Compared to other preparation methods, electrospinning technology can easily produce nanostructured extracellular matrices and controllable mechanical properties and architecture, which can provide a better environment for cell and tissue growth [45].

B. Weaving Method

The continuous fiber woven structure can be obtained by three-dimensional weaving method, which adopts continuous interweaving of fibers to form a tight network structure, has the direction of multiaxial fibers, good mechanical strength in the weaving direction, and suitable porosity. Fang et al. wove the tussah silk and implanted it into the defect of the hind limb of the rabbit [46]. It was found that the tussah silk artificial mites can support the repair of the rabbit Achilles tendon, maintain a good mechanical strength for a certain period of time, can withstand the tendon stress required by the body, and keep the repaired tendon connected firmly at both ends.

### 9.5.4 The Effect of Cell Growth Factor on Tendon Healing

The use of growth factors that promote tissue regeneration to achieve functionalization of biological materials is of great value for the repair of tissue damage [46]. By specifically binding to receptor molecules, growth factors activate downstream signaling pathways that promote cell proliferation, inhibit apoptosis, mobilize in vivo cells chemotactically, and induce stem cell differentiation and maturation. The stimulating effect of growth factors on cells is reversible and dose-dependent. It can promote the repair of damage by controlling the types and quantity of growth factors for the physiological and pathological characteristics of different lesions. The process of tendon repair, that is, the process of proliferation, migration and secretion of extracellular matrix of tendon cells, is accompanied by the release and participation of various growth factors in the process of wound repair. Cell growth factor is a biologically active protein or polypeptide

substance secreted by cells, which has the function of regulating inflammatory cell directional movement, wound cell division activation, neovascularization, and intercellular substance synthesis.

Cell growth factors associated with tendon injury repair include bone morphogenetic protein-12 (BMP-12), cartilage-derived morphogenetic protein (CDMP), platelet-derived growth factor (PDGF), vascular endothelial growth factor (VEGF), insulin-like growth factor-1 (IGF-1), transforming growth factor-β (TGF-β), basic fibroblast growth factor (bFGF), and the like [47–50]. In recent years, these cell growth factors have been applied to animal experiments to promote tendon healing, improve the mechanical properties of tendons, and reduce tendon adhesion, and have achieved positive results.

The application of cell growth factor and transgenic technology provides a new idea for tendon healing and prevention of postoperative tendon adhesion, and has important guiding significance for the clinical application of tendon tissue engineering. However, different cell growth factors play different roles at different times and different tendon healing sites, a series of questions such as how to choose the most optimal growth factor and regulate its role in the proliferation and differentiation of tendon cells, how to properly regulate the concentration of cell growth factors, when to use what kind of cell growth factor, and how to interact with each other need to be further solved. Although the single application of cell growth factor can have a positive effect on tendon repair, the effect is limited, the combined application can meet the needs of the real internal environment of the body, and help to play a synergistic effect between multigene products and improve the therapeutic effect. Therefore, the synergistic application of multiple growth factors to treat tendon injury will become a trend, and controlled release vectors and low-toxic and efficient transgenic vectors for different factors will also become the focus of future research.

## 9.5.5 Composite Culture of Tendon Cells and Scaffold Materials

### 9.5.5.1 Non-mechanical Load Culture

Non-mechanical load culture includes three-dimensional cell tissue culture and cell coculture.

A. Three-Dimensional Cell Tissue Culture
   The scaffold material having a three-dimensional structure is cocultured with different kinds of cells in vitro, and the cells migrate and grow in a three-dimensional spatial structure of the vector to form a three-dimensional cell-scaffold complex. The technology not only retains the material and structural basis of the cellular microenvironment in vivo, but also exhibits the advantages of cell tissue culture and the controllability of conditions, and is widely used. Herchenhan et al. used tendon cell composite fibrin glue scaffold to form tendon-like tissue in three-dimensional culture in vitro for 3 weeks. The mechanical strength of the cell-scaffold complex increased with the increase of fiber diameter. The changes in cytoskeleton and cell biological behavior are crucial for its function. Three-dimensional culture makes the distribution and morphology of tendon cytoskeleton beneficial to cell proliferation and growth, and regulates cell growth to approximate living tendon. During three-dimensional culture, the cells will produce sustained tensile stress, but this tensile stress is too small to be accurately determined, or it may be a stimulatory signal that plays a role in the process of cell growth along the fibers.
B. Cell Coculture
   It can maximize the simulation of the in vivo environment and facilitate the observation of cell-cell interactions. This technique has been widely used in stem cell induction, cartilage tissue engineering, and bone tissue engineering. Wang et al. constructed an indirect coculture system of BMSCs and autologous tendon cells in vitro. The results showed that the culture environment indirectly cocultured with tendon cells could induce BMSCs to express type I collagen, but no obvious expression of tenomodulin was observed. Although the application of cell coculture technology to tendon tissue engineering conditions is not yet mature, it has certain feasibility.

### 9.5.5.2 Mechanical Load Culture

The morphological structure, growth, proliferation, differentiation, and function of cells are closely related to the mechanical environment in which the cells are located [51]. They are mainly divided into compressive stress, tensile stress, shear stress, and microgravity. Tendon is a tissue that is subjected to tension. Mechanical stimulation can enhance the secretion of tissue collagen, which is beneficial to the directional growth of cells. It plays a positive role in the culture of cell-scaffold complex, stimulates the secretion of extracellular matrix, and promotes the directed growth and differentiation of cells. The addition of mechanical stimulation factors in the cell-scaffold complex culture has become a research trend in tendon tissue engineering.

A. Static Mechanical Load
   The tissue engineering tendon constructed in vitro tends to lose its initial strength, and it is difficult to maintain its inherent mechanical properties after transplantation in vivo. Therefore, enhancing the initial strength of tissue engineering tendon is one of the important contents in in vitro culture. Some scholars used collagen as a scaf-

fold material to investigate the effects of porosity, length, and mechanical stimulation on the structural strength of the scaffold. The results showed that the mechanical structure was stimulated and the longer scaffold structure had better mechanical strength. Deng et al. implanted human epidermal fibroblasts on PGA scaffolds and used a spring scaffold to form a "U" shape as a static mechanical force bearing experimental group. Compared with the nonmechanical bearing group, it can form more mature tissues and form longitudinal fibers. It secretes collagen and has good mechanical properties. Some researchers have analyzed that the tensile stress causes the cytoskeleton to stretch, which is the cause of cell morphological changes. This tensile stress acts directly on cell surface receptors or ion channels, promotes cell proliferation, and improves the transport of nutrients. It has also been suggested by researchers that cells are conformed to deformation on the nonloaded scaffold in order to bond the scaffold, so that tensile stress is generated, but the loaded scaffold may cause the cells to not produce such an active action for the force of the scaffold.

B. Dynamic Culture

Dynamic culture provides periodic mechanical strain by mechanical devices, stimulates cell directional growth on scaffolds, promotes the secretion of type I collagen, and enhances the exchange of nutrients and metabolic waste. The use of intermittent dynamic culture not only retains the advantages of dynamic culture but also increases the degree of cell infiltration and maintains the formation and retention of extracellular matrix. Qin et al. found that after applying specific stress conditions to the tendon cell-scaffold complex, the number of tendon cells, DNA synthesis, and collagen secretion were significantly higher than those of the static culture control group, indicating that periodic mechanical strain can promote tissue engineering. In the construction of tendons, Abousleiman et al. encapsulated type I collagen MSCs combined with decellularized human umbilical vein stents and gave them periodic tension with a tendon stimulator. The tissue engineered tendon formed by this method has a similar natural tendon morphology and the strain value is within the range of normal human tendon.

Bioreactor is a device commonly used for dynamic culture. It can simulate the internal environment, closely control the culture and operating conditions, has high repeatability, strong control over specific conditions, and high controllability. It can provide a suitable environment for cell to proliferate, differentiate, and biochemically react. Chen et al. combined human embryonic stem cell (hESCs)-derived MSCs into scaffolds to provide in vitro mechanical stimulation through bioreactors. hESCs-MSCs were similar to tendon cells, and cell-scaffold complexes showed good mechanical properties in the experiment of tendon regeneration after in situ transplantation. Saber et al. prepared a tendon-decellular tendon complex that was stretched using a self-made bioreactor to increase its strength and modulus of elasticity. They believed that mechanical stimulation could not directly increase the strength of the scaffold and promote the formation of ordered collagen fibers. Instead, these effects are obtained by some kind of cellular reaction. The application of bioreactor can promote cell-scaffold complex to express tendon cell-related markers and some mechanically perceptible structures and molecules in vitro, and produce nonimmunogenic tendon materials with strong mechanical properties, which has certain clinical significance.

Another method of providing dynamic mechanical stimulation is to implant a cell-scaffold complex into the skin of an animal. The subcutaneous tissue environment lacks blood vessels and has low partial pressure of oxygen, which can also provide natural dynamic mechanical stimulation, simulate tendon development and internal environment, and contribute to tendinization of cell-scaffold complex. Wang et al. constructed a human embryonic extensor tendon cell-PGA scaffold complex and designed two experimental groups of dynamic mechanical load of in vitro bioreactor and natural dynamic mechanical load on the fascia of nude mice. The results showed that the internal load group formed a larger tissue volume, the collagen fibers matured and ordered, and the mechanical properties were stronger. Therefore, it is considered that the internal load is a good method to optimize the function of tissue engineering tendon, which can make it more mature and more complete.

C. Mechanism of Mechanical Stimulation

The environment in which the cells and scaffolds are located will greatly affect the biomechanical stimulation that the cells are subjected to via the scaffold [52]. The mechanism of how the specific cellular response is affected by tensile stress and the flow rate in the culture environment is still unclear. In different combinations of cells and scaffold types, different loading methods can have different effects. The specific combination of stent tensile stress and fluid shear stress may dominate the phenotype of a certain type of cells.

Stress-strain stimulate cell metabolism and affect cell function at different levels of gene transcription, translation, cell interaction [53]. Stress-strain stimulation can not only increase the cell proliferation rate but also cause calcium ion influx to enhance the sustained secretion of proteins in cells. Stress-strain stimulation leads to assembly of integrins and organization of cytoskeleton in cells. Assembly of integrins is associated

with a variety of intracellular signaling pathways such as FAK and RhoA/ROCK signaling pathways, which in turn mediate cell differentiation and play a role in its function. Defining the role of stress in the cell signaling pathway can promote the modeling of mechanical stimulation and improve the tendon tissue engineering.

### 9.5.6 Clinical Trial of Tendon Tissue Engineering

Trauma, exercise, chronic strain, etc. can cause damage to the tendon (ligament), causing dysfunction and even disability. Tendon (ligament) injury is a common surgical injury. According to statistics, more than 300,000 patients in the United States need to undergo tendon (ligament) repair surgery every year. The demand for tendon (ligament) transplantation in China will be at least 3–4 times that of the United States in terms of population.

At present, the clinical application of tissue engineered tendon is still less reported in domestic and foreign literatures, and no product has been approved for listing.

We have been working on tendon repair materials and tissue engineered tendon since 1988. A large number of basic and applied research have been carried out on the ultrastructure of tendons, blood supply, biological characteristics of tendon cells, tendon repair materials, and the safety and effectiveness of tissue engineered tendon. In 2005, we signed a contract for the transfer of proprietary technology with the cooperative enterprises, and successively completed the product quality standards, the construction of the production quality system, the GMP assessment, and so on. Since 2007, a multicenter clinical trial has been carried out and the clinical trial was completed in 2013 and the new product registration technology review was conducted. In April 2016, the "Allogeneic Tendon Repair Materials" was approved in the National Class III Medical Device Product Registration Certificate, becoming the first approved tendon repair material product in China.

In summary, the repair and regeneration of tendon still has many unexplored areas, and tendon tissue engineering is one of the important methods of tendon regeneration. However, in many aspects, further research is needed, such as the optimization of scaffold materials, the degradation of materials, and the synchronization of extracellular matrix secreted by cells; exploring the source of clinically useful seed cells, rapid cell amplification techniques, prevention of cell senescence, and control of cell immunological reaction; improving the mechanical properties of tissue engineered tendon, making it closer to normal human tendon; tissue engineered tendon construction technique and timing of implantation in vivo; the outcome of tendon cell after implantation in vivo and its relationship with growth and development; preservation, transportation, and resuscitation of cell tissue engineered tendon products; and medical ethical issues in tissue engineering. It is necessary to further clarify the basic theory and mechanism of tendon tissue engineering. After strict quality testing and multicenter clinical verification, it can be promoted and applied clinically after approval by CFDA. With the development of cell biology, molecular biology, immunology, and materiology, and improvement in cell culture technology and methods, the combination of these fields will eventually provide a good way for tendon regeneration.

## 9.6 Tissue Engineered Myocardium

According to statistics, there are more and more patients with ischemic heart disease, and the incidence rate is increasing year by year [54]. Acute and chronic ischemia of the myocardium can lead to myocardial cell death, and severe damage leads to heart failure. At present, clinical treatment of heart failure is mainly based on drug treatment and interventional therapy. These treatment methods can delay the development of the disease to a certain extent, but cannot completely compensate for the dead myocardial cell. In recent years, with the continuous breakthroughs in research in the fields of stem cells, biomaterials, and bio-manufacturing engineering, the technology of engineered cardiac tissue has become increasingly mature. Artificial myocardial tissue is constructed to repair and replace necrotic myocardial tissue, providing a new treatment strategy for ischemic heart diseases.

### 9.6.1 The Selection and Application of Seed Cells

Considering the limited ability of terminal differentiated myocardial cells to regenerate, the main source of seed cells currently used for myocardial tissue engineering are mainly stem cells with different developmental stages and ability to differentiate into myocardial cells, including embryonic stem cells (ESCs) induced pluripotent stem cells (iPSCs) white adipose-derived mesenchymal stem cells (ADSCs), brown adipose-derived stem cells (BADSCs), and the like. In addition, myocardial cells obtained by directional transdifferentiation based on lineage reprogramming techniques are also a research hot spot in recent years. The following is a brief introduction to the characteristics of various types of cells and their application in myocardial tissue engineering.

#### 9.6.1.1 Embryonic Stem Cell

ESCs have multidirectional differentiation potential that can be induced to differentiate into three germ layer cells in vitro and further differentiate into a variety of terminal cells with single function. ESC can differentiate into myocardial cell. Researchers such as Wang conducted in-depth ESC-based myocardial tissue engineering studies, optimized ESC and embryoid body culture systems, and achieved ESC embryoid body batch amplification and myocardial differentiation. In recent years, researchers have found that ESC plays an important role in miRNA, transcription factors, and epigenetic modifications during myocardial differentiation [58, 59]. In addition, the researchers also found that transcriptional regulon CITED2, GATA4, SHOX2, Nkx2.5, etc. play an important role in the differentiation of ESC into myocardial cell [60]. Since ESC can be induced to differentiate into the cardiac muscle by signaling molecules, growth factors, small molecules, etc., ESC can be used as an important cell type for engineering myocardial tissue construction and in vivo transplantation studies. However, ESCs currently have significant limitations in transplantation applications. On the one hand, the source of ESC is ethically difficult to accept; on the other hand, the risk of immunological rejection and teratoma formation is also an important factor hindering the clinical application of ESC.

#### 9.6.1.2 Induced Pluripotent Stem Cells

Induced pluripotent stem cells (iPSCs) are a class of pluripotent stem cells that are reacquired by reprogramming somatic cells and have biological characteristics highly similar to ESCs. In 2006, Yamanaka first used retrovirus to overexpress four transcription factors OSKM (Oct4, Sox2, Klf4, and c-Myc) into mouse fibroblasts, and successfully induced the formation of iPSC [61]. Since then, researchers have developed a variety of safe and effective somatic cell reprogramming methods for the preparation of iPSC, such as the introduction of somatic cells into free plasmids, microRNAs, synthetic mRNA, recombinant proteins, and small molecule compounds. iPSC has the potential of multidirectional differentiation, but it avoids the ethical issues in ESC research and has better clinical application prospects. Mauritz and Zwi's research team have confirmed that iPSC can be induced to differentiate cardiomyocytes in vitro, and exhibit similar characteristics to ESC-derived myocardial cells in terms of cell morphology, molecular level, and electrophysiological function. In 2013, researchers such as Wang conducted research on the immunogenicity of iPSC in the microenvironment of myocardial infarction and the tumorigenicity of its differentiation products, and found that the immunogenicity of iPSC may increase with its differentiation in vivo, and the purification of iPSC-induced differentiation of cardiomyocytes before transplantation can effectively reduce the risk of tumorigenicity [62].

#### 9.6.1.3 Mesenchymal Stem Cells

MSCs has the advantages of abundant sources and simple methods of obtaining materials, which can better meet the needs of clinical applications. MSC can be differentiated into myocardial cells by induction in vitro. Researchers confirmed that MSC can spontaneously differentiate into myocardial cells in vitro, and further confirmed that MSC autocrine vascular endothelial growth factor (VEGF) plays a key role in spontaneous differentiation into cardiac muscle [63]. In vivo studies have shown that MSC transplantation has a significant effect on improving cardiac function [64].

#### 9.6.1.4 Brown Adipose-Derived Cardiac Stem Cells

In 2003, researchers discovered that enzymatic digestion of brown adipose can isolate stem cells with high myocardial differentiation for the first time, thereby expanding the source of seed cells constructed by tissue engineered myocardium. However, this method is less efficient. In 2009, Wang et al. established a method based on the "Cocktail" digestive enzyme to extract brown adipose CSC. This method improves the separation efficiency of CSC and the viability of isolated cells. The differentiated myocardium has similar characteristics to natural myocardium [65]. The above findings laid a good foundation for the application of brown adipose-derived CSC in myocardial tissue engineering.

#### 9.6.1.5 Myocardial Cells Derived from Somatic Cells

Lineage-specific transcription factors can reprogram terminally differentiated cells into other types of somatic or progenitor cells. By directly programming somatic cells to generate cardiovascular lineage precursor cells, the intermediate transition process of iPSC is not required, which greatly reduces the risk of tumorigenicity. This strategy has become a new model for the development of myocardial tissue regenerative medicine. In recent years, various new nongene integration pathways have been explored to achieve cell lineage reprogramming, such as free plasmids, recombinant proteins, microRNAs, synthetic mRNAs, small molecule compounds, and the like. The development of lineage reprogramming technology provides a safer and broader source of cells for the construction of engineered myocardial tissue.

### 9.6.2 The Selection and Application of Scaffold Materials

Scaffold material is another core component of constructing engineered myocardial tissue. Scaffold materials for myocardial tissue engineering studies are broadly classified into

natural scaffold materials and artificial synthetic scaffold materials. In recent years, new scaffold materials such as conductive nanomaterials and polymeric conductive polymers have become the frontiers and hot spots in the field of myocardial tissue engineering.

### 9.6.2.1 Natural Biomaterial

The natural biomaterial is derived from the extracellular matrix (ECM) component of natural tissues or organs; it has good biocompatibility and can serve as structural support of the tissue-specific microenvironment, and provides a good place for cells to grow and develop. Therefore, the natural biomaterial can be widely used in the field of tissue engineering research.

A. Collagen

Collagen is the main protein component of mammalian ECM, and it has good biocompatibility, degradability, plasticity, and porosity. In 1999, Simpson et al. first covered a layer of neutral type I collagen on the surface of the culture dish. The neonatal rat myocardial cells cultured on the collagen surface were arranged longitudinally along the collagen to form myocardial tissue like structure [66]. In addition, many researchers have grafted some chemical functional groups onto collagen in order to obtain specific functional properties and improve the adhesion or growth ability of myocardial cells on the scaffold material. In 2016, researchers such as Lin demonstrated that the stiffness of collagen scaffolds can be modulated by varying the degree of cross-linking, which in turn affects the differentiation of MSCs into cardiac progenitor cells.

B. Chitosan

Chitosan is a natural linear biopolysaccharide, which is widely used in tissue engineering research because of its excellent biocompatibility, good bioadhesion, and controlled biodegradability. In 2003, researchers such as Fujita used photosensitive chitosan hydrogel to control bFGF release to induce angiogenesis in the myocardial infarction site, protecting the damaged myocardium [67]. Due to the abundant grafting reaction of a large number of reactive functional groups on the surface of chitosan, the functionalized chitosan material has more mimic myocardial structure and functional properties. Another method for modifying temperature-sensitive chitosan is by grafting RoY peptide. The grafted chitosan-RoY can significantly promote cell survival, proliferation, and neovascularization under hypoxic condition; it is an ideal scaffold material for myocardial infarction repair (see Fig. 9.8) [68].

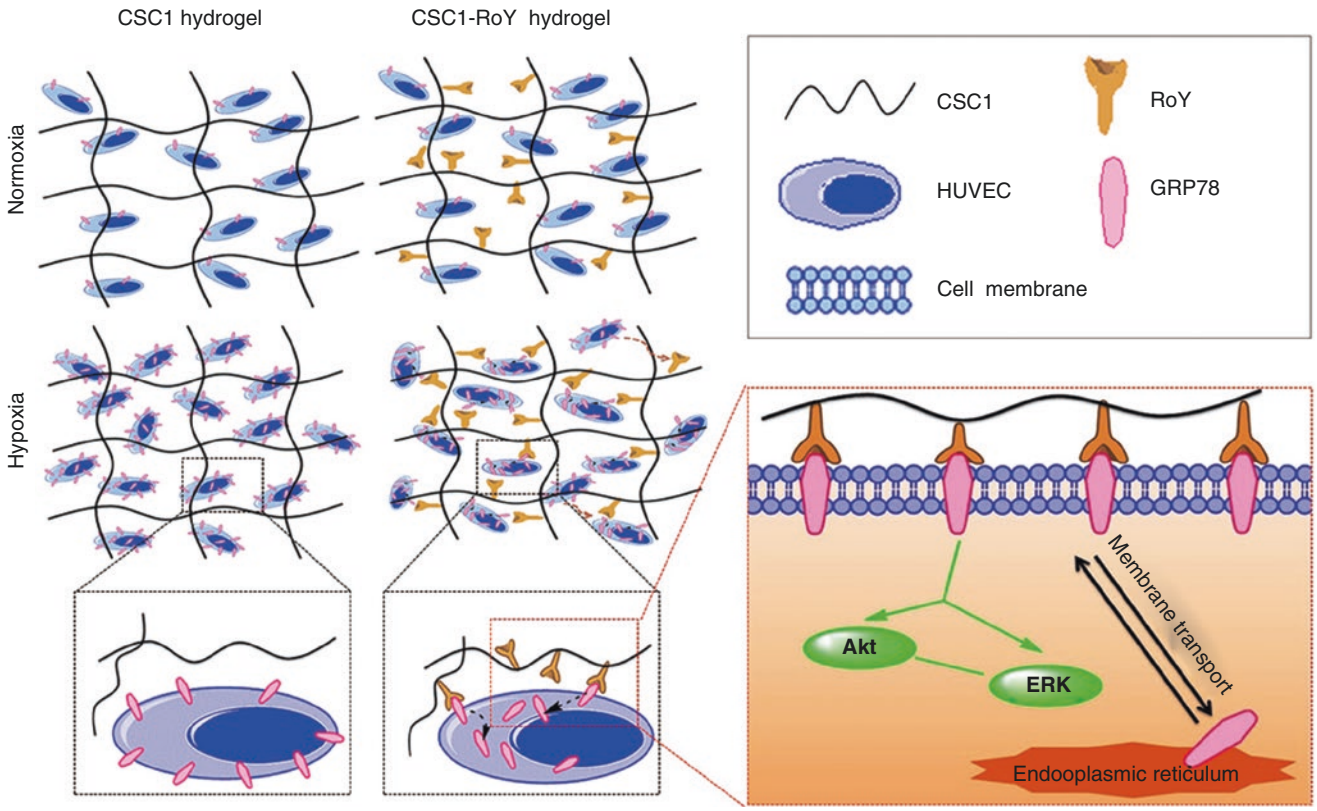


**Fig. 9.8** CSCl-RoY promotes neovascularization by stimulating the Akt-ERK1/2 signaling pathway

C. Acellular Matrix

The acellular matrix scaffold material is a three-dimensional bioscaffold material, which is obtained by acellular treatment through the combination of physical, chemical, or enzymatic methods while retaining the extracellular matrix components of tissues and organs. The main components of ECM include extracellular matrix proteins (type I collagen, mucopolysaccharide, fibronectin, and laminin) and various growth factors. In recent studies, researchers believed that ECM's ultratopological structure and 3D ultramicro conformation provide cells with "information coding" that directly or indirectly regulates cell behavior, including cell adhesion, proliferation, and directed differentiation, but specific regulation and control mechanism needs to be further studied [69].

The cardiac acellular matrix is subjected to enzymatic treatment to prepare a matrix hydrogel. In 2009, Singelyn et al. first measured the composition of the hydrogel, including type I collagen, mucopolysaccharide, fibronectin, laminin, etc., and proved its good gel-forming properties and biocompatibility, showing its enormous potential for clinical application.

#### 9.6.2.2 Artificial Synthetic New Conductive Scaffold Materials

Artificial synthetic scaffold materials mainly include traditional nonconductive and new conductive scaffold materials. Artificial synthetic traditional nonconductive materials are more focused on simulating extracellular matrix components, physicochemical properties, and spatial structure. Considering the specific structure and electrophysiological properties of myocardial tissue, it is necessary to meet the need of synchronous excitation-contraction coupling of reconstructed myocardial tissue, which further supports the formation of good structural and electrophysiological integration with the in vivo myocardium after transplantation. In recent years, artificial synthetic new conductive materials have been used as scaffold for the construction of engineered myocardial tissue. A brief introduction to new conductive nanomaterials and polymeric conductive polymers is now available.

A. New Conductive Nanomaterials

Conductive nanomaterials mainly include carbon-based nanomaterials such as carbon nanotubes (CNT), graphene, and fullerene ($^{60}$C), which have become a research hot spot in the construction of engineered myocardial tissue due to their good electrical conductivity.

Carbon nanotubes are a cylindrical sheet structure with excellent mechanical strength, electrical conductivity, and thermal stability. CNTs can be prepared into composite materials by different methods and can increase the solubility of CNTs, improve biocompatibility, reduce cytotoxicity, and greatly improve the mechanical properties and electrical conductivity of composites. Compared with simple collagen, the surface roughness of collagen/SWCNT hydrogels is significantly increased, and it has excellent electrical conductivity, good cell compatibility, and tissue compatibility (see Fig. 9.9) [70].

Graphene is a hexagonal planar thin film of honeycomb lattice made up of carbon atoms with sp2 hybrid orbitals, and has the strongest mechanical properties to date. In 2013, Kim et al. reported that graphene was used to prepare thin films and evaluated the biocompatibility and cell behavior of myocardial cells. It was found that it had good biocompatibility and had the function of maintaining myocardial cell survival, promoting adhesion and contraction [71]. Researchers used the two-dimensional

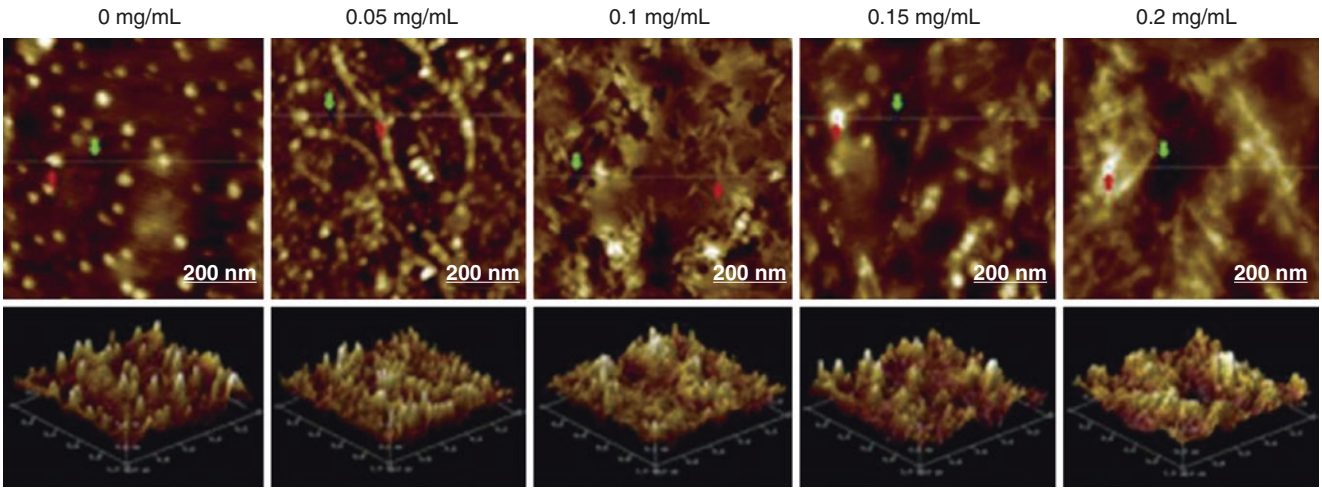


**Fig. 9.9** Development and evaluation of SWCNTs/collagen composite scaffold

sheet structure of graphene to fabricate a functionalized graphene three-dimensional tissue construct by assembly, which promoted the connection between myocardial cells and enhanced the thickness of three-dimensional myocardial tissue [72].

B. Polymeric Conductive Polymer

A polymeric conductive polymer is a type of polymer material in which a conductivity of a π bond conjugated polymer having a certain chain length is chemically or electrochemically doped to extend from an insulator to a conductor. At present, polymeric conductive polymers represented by polythiophene, polyphenylacetylene, polyaniline, polypyrrole, etc. have become one of the focuses of the field of biomaterials and tissue engineering. Polythiophene and its derivatives have good electrochemical stability, high doping level, and reversible doping and dedoping processes, which play an important role in conductive polymers.

### 9.6.3 In Vitro Construction of Engineered Myocardial Tissue and Its Role in the Treatment of Myocardial Infarction

The development of myocardial tissue engineering depends not only on the development of scaffold materials and seed cells but also on the construction techniques of engineered myocardial tissue. At present, the strategies for the construction of engineered myocardial tissue in vitro and myocardial infarction mainly include the following:

(a) In vitro construction of three-dimensional engineered myocardial tissue and treatment strategy of "band-aid" myocardial infarction.
(b) Strategy of injectable myocardial tissue engineering.

#### 9.6.3.1 Three-Dimensional Engineered Myocardial Tissue Construction Based on the Treatment Strategy of "Band-Aid" Myocardial Infarction

Engineered myocardial patch refers to engineered myocardial tissue constructed by combining seed cells with the ability to form myocardial cells and appropriate scaffold materials according to engineering principles, for in vivo transplantation to repair or replace damaged myocardium. Engineered myocardial patch can provide suitable microenvironment and structural support for seed cells, promote differentiation of transplanted cells, and prevent apoptosis.

A. Construction of engineered myocardial tissue based on dynamic mechanical stretching technique: Myocardial cells are seeded inside the material and subjected to continuous and dynamic mechanical stretching, which mimics the mechanical properties of normal myocardium and can induce myocardial cells to form a linear alignment within the material similar to that in the body. The engineered myocardial tissue constructed under this system not only has a structure similar to that of the natural myocardium, but also has mechanical strength similar to that of normal myocardial tissue, and has electrical conductivity [73]. In the same year, Wang et al. used collagen/Matrigel as a scaffold to design a mechanical device and ESC-derived myocardial cells as seed cells to successfully construct engineered myocardial tissue with spontaneous contraction in vitro.

B. Construction of Engineered Myocardial Tissue Based on Cell Layer Overlay Technology

Cell sheet technology is the use of melting membrane technology to construct a single layer of cardiomyocytes and layers of cells superimposed to form a three-dimensional cell sheet. Japanese researchers Shimizu et al. first invented a new method that did not rely on scaffold materials to support in 2002 [74]. The researchers cultured suckling mouse myocardial cells on a thin layer of temperature-sensitive polymer polyisopropylacrylamide. When the temperature was lowered, the change in the polymer separates the cell layer from the entire surface of the material. On this basis, the researchers used a variety of cell types to construct cell sheets without scaffolds, and further used for myocardial infarction transplantation studies. In the study of optimizing the technique of myocardial layer construction, in 2006, Shimizu's team successfully constructed a myocardial reconstructed tissue formed by superimposing 30 layers of myocardial slices with a maximum thickness of 1 mm. However, it is not suitable for clinical use due to thickness limitations. In order to construct a tissue that overcomes the limits of engineered myocardial thickness, it is necessary to find a way to form an adequate vasculature in vitro. In 2015, based on the layer-by-layer stacking technology, Sakaguchi developed a bioreactor perfusion vascular bed system to construct a network of blood vessels that supply oxygen and nutrient molecules similar to capillaries in natural tissues and remove waste [76].

C. Construction of Engineered Myocardial Tissue Based on Three-Dimensional Porous Scaffold Material

The three-dimensional porous scaffold material can support the adhesion and growth of seed cells.

Researchers like Roberts used collagen as a scaffold to inoculate ESC with stromal cells in collagen to construct engineered myocardial tissue [77]. Studies have shown that effective control of engineered myocardial tissue microstructure can form more complex character-

istics, laying the foundation for large-scale engineering myocardial tissue regeneration.

In recent years, the construction of engineered myocardial tissue with acellular matrix as scaffold has become a research hotspot. The engineered myocardial patch based on cardiac acellular matrix-fibrin hydrogel can effectively promote the secretion of growth factors and the growth and differentiation of stem cells, and significantly promote the formation of new blood vessels in myocardial infarction. After transplanting the constructed myocardial patch into the body, the myocardial infarct size can be significantly reduced, and myocardial function after myocardial infarction can be improved.

#### 9.6.3.2 The Construction of Engineered Myocardial Tissue Based on the Treatment Strategy of Injectable Myocardial Infarction

Injectable myocardial tissue engineering refers to the local support and microenvironment improvement of myocardial infarction by injecting the injectable scaffold material alone or carrying seed cells, growth factors, etc., and further improving the cell survival rate. Therefore, it has a good therapeutic effect on myocardial infarction. This strategy has the advantages of no need to prepare special scaffold in vitro, simple operation, less trauma to patients, and is easier to be applied in clinical practice.

In case of researching, in vitro construction of injectable myocardial tissue is based on natural matrix hydrogel chemical modification and myocardial infarction transplantation repair. In 2016, Wassenaar et al. used a porcine ventricular acellular matrix to prepare an injectable hydrogel and injected it into the myocardial infarction site of rats [78]. The results showed that hydrogel injection can significantly reduce the inflammatory response and promote neovascularization in the myocardial infarction area. It has obvious therapeutic effect on the recovery after myocardial infarction, and lays a foundation for further clinical application of acellular matrix.

In addition, the strategy of compositing cells with injectable scaffold gets a lot of attention. In 2014, Wang et al. used chitosan hydrogel as a carrier to carry a BADSC for the treatment of myocardial infarction in rats [79]. In 2014, researchers like Wang used PNIPAAm/SWCNTs hydrogel as an injectable scaffold material and carried brown adipose cardiac stem cells for myocardial infarction [80]. The study showed that after transplantation, the retention rate of brown adipose cardiac stem cell can be increased, the thickness of ventricular wall can be increased, the area of ventricular infarction can be reduced, and the cardiac function after myocardial infarction can be improved.

### 9.6.4 Cardiac Reconstruction Based on Whole-Organ Acellular–Recellular Technique

At present, the method of completely treating end-stage heart failure is the orthotopic transplantation of cardiac organs. However, due to the limited supply of donor organs, the annual demand for cardiac organs far exceeds the number of donations. In addition, after a heart transplant, individuals may also face rejection reactions such as lifelong immunosuppression. The re-engineering of artificial hearts has brought hope to solve these problems. Since the acellular matrix retains tissue-specific matrix protein components, three-dimensional ultramicro conformations, vascular structures, and mechanical properties similar to those of natural tissues, it is an ideal natural scaffold material for cardiac remodeling.

Although the research on cardiac remodeling based on whole-organ acellular matrix has progressed rapidly in recent years, there is still a big gap between the functional level of reconstructed heart and the natural heart. The first problem we need to solve is to explore the regulation mechanism of the whole-organ acellular matrix microenvironment on the fate of stem cells, so as to get closer to achieving the ultimate goal of cardiac remodeling.

### 9.6.5 Prospects and Outlooks

With the aging of the world's population, the number of people dying from heart disease each year has reached 20 million, the direct cost of heart failure treatment is as high as $120 billion every year, and the 5-year mortality rate is about 50%. The treatment of heart disease has become a heavy burden on China's national economy, which has restricted the development of the country's economy and society to some extent.

The ultimate goal of myocardial tissue engineering is to produce functional healthy heart tissue in vitro, which can be directly applied to clinical treatment, replacement of damaged myocardial tissue, or drug screening. Myocardial tissue engineering is a relatively young and rapidly developing field, but it has attracted the attention of many scientists at home and abroad. We believe that further clarification of the underlying mechanisms will continue to drive clinical practice. At present, myocardial tissue engineering has made great progress in basic research, clinical application, and market transformation, and its research results have largely changed the clinical treatment ideas and benefited a large number of patients.

Compared with cell therapy, myocardial tissue engineering treatment is complex, and it is necessary to integrate multiple factors and ultimately achieve the characteristics of

the natural heart in terms of function, cell, molecule, structure, and so on. Further research on myocardial tissue engineering relies on the synergistic development of seed cells, scaffold materials, in vitro construction of engineered myocardial tissue, and in vivo transplantation techniques. The in vitro construction of myocardial tissue provides great opportunities for regenerative medicine, drug screening, and myocardial disease models, as well as the enormous challenge of achieving reconstituted cardiac transplantation goals, relying on the cooperation and unremitting efforts of experts in the fields of biological sciences and engineering sciences. In short, there are still many unresolved problems in the field of myocardial tissue engineering, and we have a long way to go. We will wait and see.

## 9.7 Tissue Engineered Cartilage

### 9.7.1 Regeneration of Tissue Engineered Articular Cartilage

#### 9.7.1.1 The Histological Characteristics of Natural Articular Cartilage Tissue and the Epidemiology of Injury

Articular cartilage is widely distributed in the synovial joints of the human body and plays an important role, including counteracting mechanical load, lubricating joints, absorbing shock, endochondral ossification, and providing structural support. Cartilage tissue is a highly specialized connective tissue characterized by the lack of blood vessels, nerves, and lymphatic vessels in the tissue. The extracellular matrix is dense and solid, and the chondrocytes are surrounded by a large number of cell matrix (such as collagen fibers and proteoglycans). It is the main reason that the histological characteristics of cartilage tissue, the composition of biochemical components and the characteristics of low metabolism and low proliferative state of chondrocytes, which restricts the proliferation reaction of chondrocytes and the migration to the injured area, resulting in limited self-healing and regeneration capability after the cartilage tissue is damaged. Therefore, once the articular cartilage is damaged or partially excised, there is generally no direct cartilage regeneration, but the formation of connective tissue scar.

The incidence of cartilage damage is high, and it is harmful to individuals and society. According to the statistics of the National Health Commission of the People's Republic of China, there are more than 100 million patients with articular cartilage damage in China, and the number of patients is increasing by nearly ten million each year. About 90% of women and 80% of men over the age of 65 have different degrees of osteoarthritis, which is one of the main causes of labor loss in people over 50 years old. According to the International Arthritis Foundation, one out of every ten people worldwide is a patient with osteoarthritis. A large group of patients with osteoarthritis affects the quality of life of patients themselves, and it also brings huge economic burden to the society. Statistical data from the United States showed that the total cost of osteoarthritis in 1994 was $15.5 billion. From other developed countries, such as Canada, the United Kingdom, and France, the cost of osteoarthritis in 1997 accounted for 1–2.5% of the country's GDP for the year, and increased year by year. There is still no such statistical data in our country. Other ear cartilage defects and nasal cartilage injuries caused by burns, trauma, genetic factors, etc. affect the physiological, psychological, and social health of patients to varying degrees.

Current strategies for treating cartilage damage or osteoarthritis are limited and lack effective treatment options. It mainly includes conservative treatment and surgical intervention. The former includes reducing joint use, increasing muscle strength around the joint, local physical therapy and rehabilitation therapy, and using drug to relieve pain. The latter includes arthroscopic inflammation cleaning, microfracture surgery, and platelet factor enrichment (PRP) treatment, tibia osteotomy, autologous cartilage tissue transplantation (mosaic technique), autologous chondrocyte implantation (ACI), and artificial joint replacement surgery. Conservative treatment techniques, arthroscopic inflammatory cleaning, and tibia osteotomy can relieve symptoms and delay the development of cartilage damage to a certain extent, but cannot achieve the repair and regeneration of damaged cartilage tissue. The purpose of microfracture technology, PRP treatment, mosaic technology, and ACI technology is to repair the damaged cartilage tissue, and hope to achieve the regeneration of cartilage tissue. However, the newly obtained cartilage tissue is mostly fibrocartilage tissue, and its biomechanical strength is worse than that of normal hyaline cartilage tissue, and the surface of the tissue is rougher than normal tissue, which makes it unable to satisfy the function of long-term normal joint function. In addition, the source of autologous chondrocytes or autologous cartilage tissue is limited, and it will bring new cartilage damage to patients themselves. These are the dilemmas and problems that need to be solved by clinicians and researchers.

In recent years, with the rapid development of biomaterial science, engineering science, computer technology, and biomedical science, the exchanges of interdisciplinary information have become increasingly rich and extensive. As a new frontier interdisciplinary subject, tissue engineering has been proposed and widely used in the field of tissue regenerative medicine [81]. In 1987, the concept of tissue engineering was systematically proposed by the American chemical engineer Langer and the clinician Vacanti, mean-

ing the combined use of cells, scaffold materials, and bioactive factors to promote tissue repair and regeneration. The specific method is to obtain a small amount of normal tissue from the human body, obtain the target seed cells through in vitro treatment, and then amplify and plant them on the scaffold material, construct the engineered tissue and implant into the body, repair the relevant tissue defects, and restore the original function. The proposal and development of tissue engineering has broken through the traditional helplessness of repairing damaged tissue at the expense of injured autologous tissue, which brings new hope for the repair and reconstruction of cartilage defects [82, 83].

The research and development of tissue engineered cartilage can be roughly divided into three historical stages. The first phase was mainly from the late 1980s to the early 1990s. The research team led by Vacanti et al. successfully constructed hyaline cartilage tissue in nude mice with isolated bovine articular chondrocytes and degradable biomaterials. The results have successfully confirmed that tissue engineering technology can be fully used for cartilage regeneration and repair. The second stage of tissue engineered cartilage development was in the mid-to-late 1990s. It mainly studied the construction of various shapes of tissue engineered cartilage and preliminary cartilage regeneration and repair in nude mice with immunodeficiency, or in mice, rabbits, and other small animals. In this phase of research, people have begun to pay attention to the selection and application of seed cells for tissue engineered cartilage. The study of tissue engineered cartilage in nude mice and small animals cannot fully and truly reflect the interaction between the microenvironment in vivo and seed cells and biomaterials. Therefore, further research and development of tissue engineered cartilage is naturally turned to tissue engineered cartilage repair and regeneration which is constructed in a large mammal with full immune function, that is, the third stage of the development of tissue engineered cartilage research. At this stage, various studies on tissue engineered cartilage have been carried out.

#### 9.7.1.2 Important Elements and Mechanisms of Tissue Engineered Cartilage Regeneration

The three major elements of tissue engineered cartilage repair are scaffold materials, seed cells, and cytokines [84]. Seed cells are considered the core of tissue engineering. The scaffold materials are the carrier of seed cells and the framework of new tissues. Cytokines are carriers of intercellular signaling and important biologically active molecules that regulate cell proliferation, differentiation, and metabolism.

A. Scaffold

As a substitute for the extracellular matrix of chondrocytes, scaffolds play an important role in cell adhesion, growth and reproduction and secretion of normal extracellular matrix during late cartilage repair.

The ideal scaffold is a temporary carrier of cells. It should have the following characteristics in articular cartilage tissue engineering:

(a) It has good biocompatibility. The scaffold material itself or degradation products are not toxic to cells, tissues, and organisms, and do not cause immunological rejection after implantation.
(b) It is biodegradable. The rate of degradation matches the rate of tissue regeneration. It is generally believed that materials with a short degradation time are more suitable for cartilage tissue engineering than materials with long time stability, because the latter inhibits the synthesis of the matrix in the later stage of the formation of new tissue.
(c) It has sufficient pore structure and porosity and generally requires a porosity of more than 90%. It can provide enough space for even distribution and growth of cells.
(d) The surface of the material has good biological activity and can promote cell adhesion and proliferation. It can regulate the adhesion and growth of seed cells through surface modification, controlled release of biomolecules, or response to environmental stimuli.
(e) It should have the ability to carry and release growth factors while ensuring that the volume of the stent remains unchanged.
(f) The scaffold has mechanical strength and elasticity matching the surrounding normal tissue, which can meet the needs of conduction stress in early joint activities.

The tissue engineered scaffold materials currently reported and studied can be mainly divided into three categories: natural biopolymer scaffold materials, artificial synthetic degradable scaffold materials, and composite scaffold materials [85].

Natural polymer bioscaffold material: Natural bioscaffold material has the advantages of good biocompatibility, high cell affinity, and high degree of degradability, and is beneficial to the adhesion and proliferation of seed cells in the later stage, but it also has shortcomings of poor mechanical properties and rapid degradation rate. The natural biomaterials currently in clinical research mainly include collagen, fibrin, chitosan (CS), silk fibroin, hyaluronic acid, acellular cartilage extracellular matrix, alginate, gelatin, agarose, chondroitin sulfate, chitin, and the like. Extracellular matrix is a macromolecule synthesized by animal cells and secreted extracellularly, distributed on the surface of cells or between cells, mainly composed of protein, polysaccharide, or proteoglycan. Acellular cartilage extracellular matrix scaffold is

a better tissue engineered cartilage scaffold, which effectively removes the antigenicity of cells, mimics the microenvironment of chondrocyte growth, and makes this natural bioscaffold very beneficial to the adhesion and proliferation of cartilage cells and has superior biocompatibility and cell affinity. However, the disadvantage is that the natural conformation of the extracellular matrix of the cartilage is easily destroyed during the preparation process, and the mechanical properties of the scaffold are relatively poor. Collagen is the main fibrin component in the extracellular matrix of cartilage. It is usually divided into type I, II, III, IV, and V collagen. Its main function is to connect tissues and organs, support body weight, and protect the body. Experimental studies have shown that the collagen scaffold has the advantages of low immunogenicity, high hydrophilicity, good biocompatibility, cell affinity, and is beneficial to the growth and differentiation of the seed cells. However, the corresponding disadvantages are that the collagen scaffold degrades too fast, the toughness is poor, and the mechanical stress intensity is insufficient. It is usually used in combination with other materials to exert its performance, but how to solve its own related problems needs further research.

Artificial synthetic degradable scaffold materials: Due to the shortcomings of natural polymer materials, which cannot directly meet the corresponding requirements of tissue engineered cartilage, more and more researchers have turned their attention to artificial synthetic scaffold materials [86]. Artificial synthetic scaffold materials have been widely concerned because of their good mechanical properties, strong plasticity, controllable degradation rate, and unrestricted source. The corresponding disadvantages are that the biocompatibility is poor, the cell affinity is low, and the degradation products of scaffold materials may have certain toxicity.

Commonly used artificial synthetic scaffold materials mainly include polylactic acid (PLA), polyglycolic acid (PGA), polylactic co-glycolic acid (PLGA), polyoxyethylene, polycaprolactone (PCL), polyvinyl alcohol (PVA), polyepoxyethylene, and the like. Polylactic acid (PLA), also known as polylactide, belongs to the polyester family. It is a synthetic polymer material with good biocompatibility, degradable, and most of the degradation products are $CO_2$ and $H_2O$. Compared with natural bioscaffold materials, polylactic acid is not only mature in processing technology, but also has a good source of raw materials and good biomechanical properties. The intermediate product lactic acid is also a normal sugar metabolite in the body and will not have harmful effects on the body. The disadvantage of polylactic acid is that the degradation rate is too slow. Usually, the degradation half-life is 6–8 weeks. Although sufficient time provides a scaffolding effect on the cartilage repair area, the long-term accumulation of lactic acid in vivo easily causes tissue swelling and inflammation reaction, and the cell surface affinity of the scaffold material is poor, which is not conducive to cell adhesion and growth, and further limits its development in cartilage tissue engineering. Polyglycolic acid (PGA) is also a commonly used artificial synthetic material, and its degradation products are mainly nontoxic glycolic acid, and the degradation products can be excreted with the renal excretion system or further involved in the metabolic cycle. Polyglycolic acid has good histocompatibility and can promote cell adhesion, growth, and induced differentiation. However, its disadvantage is that the degradation rate is too fast, which easily leads to the collapse of the whole scaffold. The local mass accumulation of the acidic degradation product glycolic acid in vivo also causes a decrease in the pH of the surrounding tissue and a certain effect on the cells. Therefore, in the experimental study, PGA often combines with PLA to form a PLGA to improve its degradation rate to the desired level.

Composite materials: The emergence of composite materials is the development of tissue engineering. In order to overcome the shortcomings of each single material, two or more materials were combined in a certain proportion or way with complementary methods of their characteristics and advantages to design an ideal scaffold which can meet the needs of cartilage tissue engineering. The composite scaffold has the advantages of each single scaffold, such as controllable degradation rate, good cell compatibility, good hydrophilicity of the scaffold, and suitable biomechanical strength. Three-dimensional porous scaffolds with specific shape and pore structure are made by particle pore method, freeze drying method, and laser pore method, such as collagen-PLGA composite scaffold, collagen-hyaluronic acid-chondroitin sulfate composite scaffold, and polylactic acid-polyglycolic acid-calcium polyphosphate fiber-collagen composite scaffold.

B. Seed Cell

Seed cells are the basic unit of cartilage tissue engineering. The selection and optimization of seed cells is the key and prerequisite for constructing tissue engineering. The ideal seed cells should ensure that:

(a) It is safe and convenient.
(b) The sources are abundant.
(c) The immune rejection caused by the body after implantation in vivo is small.
(d) Cells have strong proliferation and differentiation ability.
(e) It can maintain a stable chondrocyte phenotype.
(f) It can adapt to the microenvironment of materials and damage repair areas.

Seed cells can be roughly divided into two major categories in the construction and application of tissue engineered cartilage: adult chondrocytes and mesenchymal stem cells [87, 88]. Chondrocytes can be further divided into autolo-

gous chondrocytes, xenogenic chondrocytes, and allogeneic chondrocytes; stem cells mainly include mesenchymal stem cells derived from bone marrow blood, adipose tissue, embryonic tissue, perinatal tissues (umbilical cord tissue, cord blood, and amniotic tissue), etc.

Chondrocytes belong to terminally differentiated cells and are one of the earliest seed cells used in tissue engineered cartilage research. They are also considered to be the gold standard for repairing cartilage seed cells. The biggest advantage of autologous chondrocytes is that there is no immune rejection, which is beneficial for direct clinical application. However, due to its limited source, the process of taking the material will cause new damage to the body, and the chondrocytes are terminal cells with very weak differentiation ability. Usually after about four generations of subculture, the cell phenotype is prone to variability and instability. Type II collagen, which is highly expressed in the extracellular matrix of the cartilage, gradually becomes type I collagen, and the growth and proliferation ability of the cells is deteriorated, thereby failing to reach the number of tissue engineered cartilage requirements, which limits its application. Xenogeneic or allogeneic chondrocytes have a wide range of sources and can be obtained and proliferated in a relatively short period of time. However, since they may produce large immune rejection, they are less practical, so they are not the most ideal tissue engineered cartilage seed cells.

Mesenchymal stem cells are regarded as one of the most promising tissue engineered seed cells because of their multidirectional differentiation potential and self-replication ability, and they have received extensive attention in the field of tissue engineered cartilage research. The stem cells used in the current research are mainly bone marrow mesenchymal stem cells, adipose stem cells, and umbilical cord stem cells. There are bone marrow mesenchymal stem cells (BMSCs) with multidirectional differentiation potential in the bone marrow. BMSCs can be taken from the bone marrow of the backbone, tibia, and ribs. The source is relatively abundant, and the acquisition method is simple and easy. BMSCs can differentiate into chondrocytes under the influence of inducing factors, and it has been recognized that it may become an ideal source of seed cells for articular cartilage tissue engineering. The study found that adipose-derived stem cells can differentiate and culture chondrocytes and stably passage. However, the study also found that after ten passages, the cells showed aging, such as increased lipid droplets, synaptic elongation, and slowed cell proliferation. Only cells within five generations are suitable for cartilage repair. In addition, cells such as periosteum, synovial stem cells, and muscle-derived stromal stem cells can also be induced to differentiate into chondrocytes, but they are less used in current research due to less sources and more complicated differentiation mechanisms. Embryonic stem cells (ESCs) are mainly derived from early embryos developed by fertilized eggs, and can also be obtained from an embryo developed from a somatic cell nuclear transfer. The ability of ESCs to be versatile and immortal is expected to be a new source of tissue engineered seed cells. Since the early 1980s, some scholars have used the inner cell mass or the epiblast cells of early embryos to establish ESCs. Some scholars have confirmed that ESCs can differentiate into chondrocytes under the action of BMP-2 and BMP-4. At present, the biggest problem in the application of ESCs is how to effectively induce the differentiation of ESCs into chondrocytes, effectively establish nonimmunogenic embryonic stem cell lines, and the tumorigenicity of ESCs. In order to solve the problem of difficult source of seed cells, scholars apply transgenic technology to articular cartilage tissue engineering, and transfect the genes of TGF-β, BMP, bFGF, IGF, and other factors into the corresponding target cells by means of vectors to differentiate them into chondrocytes. The most commonly used target cells are BMSCs. It has been reported that chondrocytes are cultured from BMSCs by transgenic technology. At present, there are three methods for introducing foreign genes into a target cell: in situ method, indirect method or ex vivo method, and direct method or in vivo method. With the deepening of research, some new inducible genes have been discovered one after another. Overexpression of SOX9 gene can promote the redifferentiation of osteoarthritic chondrocytes in alginate culture medium, and transfect BMSCs by adenovirus, and can be cultured in the cartilage forming matrix to differentiate into the chondrocyte and secrete type II collagen and the protein polysaccharide. These studies offer new possibilities for culturing cartilage seed cells that meet clinical applications. However, the current transgenic research on BMSCs in China still stays in the animal experiment stage. There are still many problems in the application of transgenic BMSCs in clinical practice, such as the problems of BMSCs transplantation in cartilage microenvironment, and whether it will cause malignant transformation and whether the original organism can maintain the original biological characteristic after transgenic, etc., are for further study.

C. Cytokines

Cytokines are important elements in tissue engineering to regulate the growth and reproduction of seed cells, and are important active substances that stimulate and promote seed cells to perform their intended repair functions in a timely and appropriate amount. The application methods in tissue engineering mainly include directly adding cytokines to the culture medium, regulating cell-directed cartilage differentiation, and performing gene integration by introducing the target gene into the recipient cells to continuously express the target factor protein in the cells. At present, the growth factors studied more include TGF-β, IGF, FGF, BMP, cartilage-derived morphogenetic protein (CDMP), etc. TGF-β is considered the most important cartilage-inducing factor and plays an important role in cell proliferation and differentia-

tion. It is widely present in the body's cells, with the largest number of osseous tissue and platelets. By regulating cell autocrine, paracrine, and other stimulation of chondrocytes to synthesize proteoglycans and collagen, and inhibiting matrix degradation, stem cells can be induced to differentiate into chondrocytes in a dose-dependent manner. IGF can promote the DNA synthesis of chondrocytes, the proliferation of chondrocytes, and maintain its phenotypic stability, and even induce the differentiation of adipose-derived stem cells into chondrocytes. After topical application of IGF-1, it was observed that the apoptosis of chondrocytes was significantly reduced, and the repair of damaged cartilage was significantly accelerated. FGF is found in many tissues such as the brain, liver, kidney, cartilage, and bone of animals and humans. The study found that the mechanism of action of FGF is to combine with the receptors of heparin mucopolysaccharide molecules in the extracellular matrix and activate cells, thereby effectively promoting cell mitosis, inducing cell morphogenesis and differentiation, and participating in the repair process of cell growth, proliferation, and tissue damage. In addition, FGF can significantly inhibit the differentiation of naive chondrocytes, reduce the synthesis of alkaline phosphatase, inhibit the absorption of calcium by chondrocytes, and thus prevent the calcification of new cartilage. BMP belongs to a subgroup of the TGF-β superfamily and has many species. Its role is to mediate early development and organ formation during embryonic formation. In the motor system, BMP promotes the development of cartilage, bone and motor system-associated connective tissue. BMP-2, BMP-4, BMP-6, BMP-7, and BMP-9 have been shown to have significant osteogenesis, while BMP-3 has an inhibitory effect on osteogenesis. Different BMP subtypes have different stimulating effects on chondrocytes, and BMP-7 and BMP-6 have important effects on the repair and regeneration of cartilage damage. CDMP includes three subtypes of CDMP-1, CDMP-2, and CDMP-3, which are very similar in terms of structure or biological characteristics. They are involved in the whole process of cartilage tissue development, growth, and damage repair. It plays an important role in the regulation of chondrocyte differentiation. Among them, people's research on CDMP-1 is the most in-depth. CDMP-1 is mainly expressed in the bone and joint areas of the extremities. It is an important regulator of early cartilage. It can promote the aggregation and adhesion of cartilage progenitor cells in a dose-dependent manner. In the late stage, it plays a role in stimulating chondrocyte proliferation and promoting its maturation.

In summary, TGF-β, IGF, FGF, BMP, and CDMP coordinate and interact with each other in the growth, development, proliferation, differentiation, metabolism, and apoptosis of chondrocytes. The repair of damaged cartilage is an extremely difficult and complicated process, and the development of cartilage tissue engineering technology can greatly promote the repair of human cartilage, and has broad application prospects. The cartilage repair process is the result of a combination of multiple cytokines. In the past, researchers paid more attention to the role of single cytokines, and often ignored the complex effects of different cytokines. The combination of different factors often showed stronger synergistic or antagonistic effects. Furthermore, the signaling pathways and dose-effect relationships of various factors also require more in-depth exploration. In addition, when cytokines have an effect on other tissues, even harmful effects, the clinical safety of cytokines needs to be further explored.

#### 9.7.1.3 Challenges in Tissue Engineered Cartilage Regeneration and the Direction of Future Conquest

Although tissue engineering research has brought new hopes for the regeneration and repair of cartilage tissue, some techniques have been applied from basic research to clinical treatment of articular cartilage damage, and have achieved gratifying therapeutic effects. However, the new cartilage tissue still has a certain gap in biochemical composition, tissue structure, and biomechanics compared with normal hyaline cartilage. However, tissue engineered neonatal cartilage tissue can relieve pain symptoms in a short period of time, filling cartilage defects and providing certain joint movement function. However, the rate of re-injury of the repaired tissue is still faster than that of normal tissue, and the long-term effect is still not satisfactory. The reasons include the following aspects:

(a) The seed cells used to construct tissue engineered cartilage did not achieve the transformation of hyaline cartilage, or lost the original hyaline cartilage phenotype after in vitro processing.
(b) The biological properties, mechanical properties, and degradation rate of the scaffold materials cannot meet the needs of the dynamic remodeling process of tissue regeneration.
(c) The cytokine's mechanism of action, added components, dose, and timing are lack of detailed and sufficient basic research evidence support.

Therefore, tissue engineered cartilage should achieve a qualitative leap, and a large number of valuable basic research and clinical transformation application research work need to be carried out. The following aspects may be the most promising and prospective research directions in the future: seed cells should be widely sourced, have low immunogenicity, have good cartilage differentiation potential and good cell biological activity, and can rapidly amplify and proliferate in vitro and maintain the phenotype of chondrocytes unchanged. In the process of biomaterial research and development, special attention should be paid to the study of the interaction between cells and biological materials to avoid the disconnection between bio-

logical materials research and seed cell research. The development of composite materials, nanomaterials, biomimetic materials, and smart materials for biological or chemical modification of materials will be an important part of future biomaterial research. The development of composite materials, nanomaterials, biomimetic materials, and smart materials for biological or chemical modification of materials will be an important part of future biomaterial research because of providing structural support and spatial guidance distribution for cells, inducing cell differentiation and promoting cell proliferation, and ultimately accelerating the regeneration and repair of functional tissues. Cytokines also play an important role in tissue engineering tissue regeneration, and play a bridge-like presentation role in signal transmission between cells [89]. In the future, more research on mechanisms may be needed to reveal its role in tissue regeneration and repair, the role of signaling pathways, and the physiological functions of cells that regulate cell development, so as to apply it more accurately and meticulously to tissue engineering to regulate the occurrence and development of target cells. The way of adding cytokines should also be presented in various ways along with the development of materials science and molecular biology, such as nanomolecular materials coated to achieve slow release, or the synthesis and release of cells with the target gene-transfected cells permanently associated with cell metabolism, so as to achieve the function of cytokines for a long time.

## 9.7.2 The Regeneration of Tissue Engineered Meniscus

### 9.7.2.1 Histological Characteristics and Physiological Functions of Meniscus

A. Anatomy of the Meniscus [90]

The meniscus is a pair of crescent-shaped fibrocartilage tissue between the femoral condyle of the knee and the tibial plateau (Fig. 9.10). The lateral meniscus covers approximately 80% of the tibial plateau, whereas the medial meniscus covers only approximately 60% of the tibial plateau. The unique shape of the meniscus fits perfectly into the different configurations of the corresponding knee femoral condyle and tibial plateau. The anterior and posterior cruciate ligaments play a very important role in the firm fixation of the meniscus, which secures the meniscus to the tibial plateau. The vascular and neural tissues of the meniscus are mainly derived from the surrounding joint capsule and synovial tissue, and there are blood vessels and nerve tissues in the adult knee joint only in the 10–25% area outside the meniscus. Therefore, according to the different distributions of meniscus blood vessels and nerve tissue, the meniscus tissue can be classically divided into three regions: vascular/neural region on the outside (red-red region), no vessel/neural region on the medial side (white-white region), and the region between the two (red-white area). Therefore, when the injury occurs in the white-white area, the meniscus injury is often difficult to heal by itself.

B. Composition and Cell Characteristics of the Meniscus

The extracellular matrix and cell distribution of the meniscus are characterized by heterogeneity. The extracellular matrix component of the meniscus is more complex than the extracellular matrix component of articular cartilage. The extracellular matrix components of articular cartilage are characterized by uniformity, mainly composed of water, collagen, and glycosaminoglycans. The extracellular matrix components of the meniscus can be classified according to different regions. Type I collagen accounts for more than 90% of the dry weight of the red-red region, and the remaining component composition is less than 1%, including collagens of type II, III, IV, VI, V, and VIII. However, in the white-white region, the total collagen content is only 70% of the dry weight, of which type II collagen and type I collagen account for 60% and 40%, respectively.

Distribution of extracellular matrix components of meniscus heterogeneity (Fig. 9.11).

The cell population of the meniscus can also be classically divided into three different species based on different

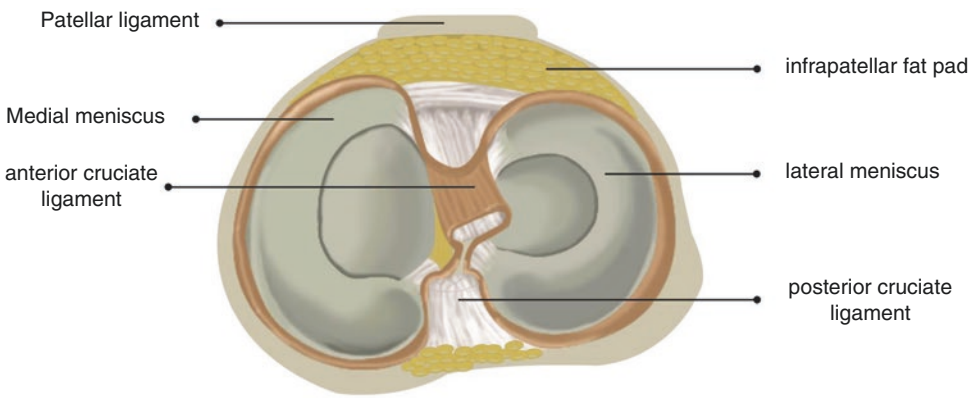


**Fig. 9.10** Anatomy of the meniscus

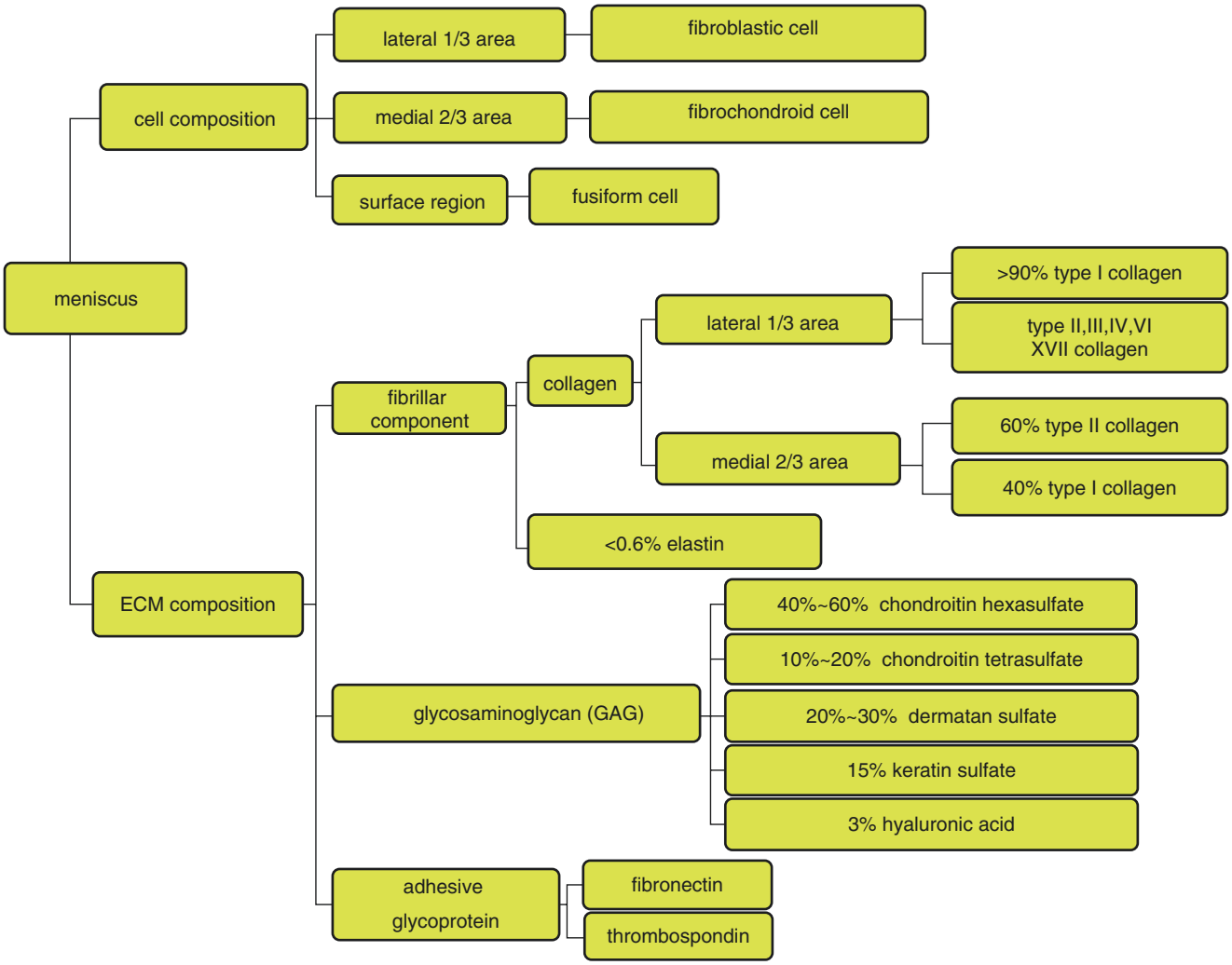


**Fig. 9.11** Composition and cell characteristics of the meniscus

regions and cell morphological features (see Fig. 9.11). The lateral one-third region of the meniscus is mainly composed of fibroblast-like cells, and mainly exhibits an elongated cell shape. The cells in the two-thirds region of the inner side of the meniscus are mainly composed of fibrocartilage-like cells and mainly exhibit circular features. The surface layer of the meniscus is composed of fusiform cells parallel to the surface of the meniscus.

C. Physiological Function of the Meniscus

The meniscus has long been considered a "non-functional residual of the origin of the thigh muscle." With the gradual deepening of the physiological function of the meniscus, people gradually discovered that the meniscus plays a very important role in maintaining the normal function of the knee joint, including weight transfer, shock absorption, stabilization of the knee joint and nutrition, lubrication of the knee joint, and other functions. The biomechanical function of the meniscus is also the basis for its physiological function. The anatomical configuration of the meniscus is closely related to the biomechanical properties of the meniscus. The special shape of the meniscus is extremely important for its adaptation to the different anatomical configurations of the corresponding femoral condyle and tibial plateau. Undoubtedly, the shape of the meniscus adapts to the increased contact area of the articular cartilage between the femur and tibial plateau in the knee joint. In daily life, while the meniscus is subjected to axial stress, it also produces cerclage stress along the semicircular direction of the meniscus, which tends to extrude the meniscus into the knee joint. However, the firm fixation of the anterior and posterior cruciate ligaments prevents the extrusion effect of the meniscus. Therefore, this ensures that a complete meniscus is located between the corresponding femoral condyle and tibial plateau and increases the contact area of the articular cartilage (60%); likewise, it can simultaneously reduce the contact stress of the tibial plateau and protect the tibial plateau cartilage. Conversely, if the

anterior and posteriorcruciate ligament or the semicircular collagen fibers in the meniscus are broken, it will change the mechanism of weight transfer and damage the tibial plateau cartilage.

#### 9.7.2.2 The Damage of Meniscus Tissue and Its Epidemiology

After meniscus injury, different degrees of degeneration, edge and surrounding muscle tissue hyperplasia, hypertrophy, edema, etc., may occur. The knee joint will lose its stability and normal activity, and there will be symptoms such as quadriceps atrophy, joint pain, squeaking, shackles, and limited mobility. Long-term meniscus injury will lead to damage to the corresponding articular cartilage of the adjacent femur and tibia, induce osteoarthritis, and lead to a decline in the quality of life and viability of patients; patients with severe disease development need to undergo joint replacement therapy.

In the United States, meniscus injury is the most common intra-articular injury and is the most common disease requiring orthopedic surgery. According to reports, the average incidence of meniscus injury is 66/100,000, and 61 per 100,000 people need to undergo meniscectomy. Men are more prone to meniscus injury than women, and the ratio of male to female is (2.5:1)–(4:1), and the peak age of onset is between 20 and 29 years. In all age groups, meniscus injury most often occurs in the right knee joint, but the main etiology and pathophysiological factors vary greatly depending on the age of the patient. Meniscus injuries in young people are more common in acute injuries, but more often in the elderly population, they are secondary to degenerative changes. More than one-third of meniscus tears are associated with anterior cruciate ligament injury. From the point of view of clinical diagnosis and treatment, meniscus injury can be easily divided into two main types of damage: peripheral meniscus injury and white-white region meniscus injury.

There is relatively little content in the epidemiological study of meniscus injury in China. Yi Shouhong and others used patients with knee arthroscopy from October 2005 to November 2010 in the Joint Surgery Center of Southwest Hospital of Third Military Medical University as the research object. The clinical epidemiological characteristics of 3002 cases of knee arthroscopy were studied. It was found that meniscus injury accounted for 48.47% of knee arthroscopic surgery, indicating that meniscus injury in China is also the most common disease in knee injury.

#### 9.7.2.3 Regeneration Dilemma After Meniscal Tissue Injury

The unique blood supply and nerve distribution characteristics of the meniscus determine that the damage that occurs in the white-white region is difficult to heal by itself. Clinically, orthopedic surgeons typically perform partial meniscal resection for patients with meniscus injuries that cannot be repaired or undergo degenerative changes. However, this current major surgical treatment cannot prevent the development of knee osteoarthritis in patients with meniscus injury, which may be due to the reduction of the contact area between the femoral condyle cartilage and the tibial plateau cartilage after meniscal tissue resection. Therefore, meniscal repair or reconstruction techniques have received widespread attention. In general, repairable injuries in young patients, such as long-axis injuries or injuries that occur in the red-red region, are the best indication for meniscal repair surgery. Meniscus injury repair procedures include internal and external, external and internal repair, and enhanced repair. On the other hand, an increasing number of meniscus reconstruction strategies have also been applied to restore meniscus function, including meniscus allograft, small intestinal submucosal transplantation, and autologous ligament transplantation. However, allografts of the meniscus also have many limiting factors, including the risk of disease transmission, the decline in biomechanical properties of the graft, and graft atrophy. Similarly, small intestinal submucosal transplantation and autologous ligament transplantation did not achieve a satisfactory therapeutic effect.

#### 9.7.2.4 Proposal of the Concept of Cartilage Regeneration in Meniscus Engineering

The current strategy of meniscus injury treatment is difficult to achieve the purpose of functional regeneration of the meniscus. The idea of tissue engineering technology and the concept of regenerative medicine have brought new hope to the treatment of meniscus injury [91]. Tissue engineering technology consists of three classic parts: cells, scaffolds, and biochemistry and biomechanical stimulation. From a tissue engineering point of view, the ideal state is to construct a functional meniscus in vitro to replace damaged or degenerated meniscus tissue. The development of materials science, the advancement of medical imaging technology, and the flourishing development of 3D printing technology have brought new hopes for the realization of the regeneration of a tissue whose shape and function can completely replace the damaged meniscus.

Nowadays, a large number of strategies have been applied to the in vitro construction of meniscal tissue engineered cartilage, which can reconstruct the meniscus defect to some extent, both functionally and structurally. There is no doubt that choosing the right source of cells (autologous, allogeneic, xenogeneic, or stem) is one of the keys to successful meniscus tissue engineering. Similarly, the research and exploration of different types of tissue engineered meniscus scaffold are also extremely critical, whether applied to experimental or clinical research. There are also inevitably some problems to be solved, such as degradation by-products of the scaffold, stress shielding, and the like. In order to pro-

mote functional meniscus reconstruction and stem cell differentiation, some cytokines have also been explored. Although there are a lot of problems in the development of meniscus tissue engineering, with the development of biology, engineering, and medicine, the research of tissue engineered meniscus will continue to be promoted.

#### 9.7.2.5 Important Elements and Mechanisms of Cartilage Regeneration in Meniscus Engineering

In the field of meniscal engineering cartilage, the regeneration of the meniscus mainly includes three important components of seed cells, scaffolds, and factors. In the research of meniscal seed cells, different institutions have carried out research on adult cells, stem cells, and co-culture of various cells. Fibrocartilage cells: the team of Professor Baker subcultured the meniscus of patients undergoing knee surgery to obtain P2 fibroblasts and implanted in polycaprolactone (PCL)-oriented scaffolds, and found that with the prolongation of culture time, the dry weight, DNA, collagen, and glycosaminoglycan content of the cell-scaffold complex gradually increased. Sun-Woong Kang from Hanyang University in South Korea transplanted a meniscus with a meniscal-derived fibrocartilage-complexed polyglycolic acid (PGA) scaffold for rabbits, and found that the new tissue is similar to the original tissue in collagen content and mechanical properties. Chondrocytes: Fibrocartilage cells and chondrocytes are derived from cartilage tissue, and studies have shown that they have similar surface markers and high expression of type II collagen. Therefore, chondrocytes are also one of the most promising seed cells for tissue engineered meniscus. To this end, Hong Suk Kwak et al. repaired meniscus injury with a polylactic acid-glycolic acid copolymer scaffold treated with chondrocyte-rich platelet-rich plasma (PRP). The results showed that chondrocytes were a viable meniscus seed cell. Mesenchymal stem cells: stem cells have multiple differentiation potentials, so they are also one of the best seed cells for tissue engineered meniscus. The University of Bristol in the United Kingdom used human bone marrow stem cells and chondrocytes to grow in collagen scaffolds for in vitro culture. It was found that the collagen scaffolds combined with transforming growth factor-β-induced bone marrow stem cells had better mechanical properties. Some scholars have also carried out research on synovial cells, adipose stem cells, and mesenchymal stem cells as seed cells of meniscus. In China, Xu Qinglei and others used fibroblast growth factor and TGF to induce bone marrow stem cells to differentiate into cartilage, looking for a seed source that can replace meniscus fibrocartilage cells. Zhu Xianqi et al. used porous silk fibroin/hydroxyapatite combined with bone marrow mesenchymal stem cells to repair cartilage damage in rabbit meniscus without blood supply area. Professor Yu Jiakuo from the Third Affiliated Hospital of Beijing Medical University used peripheral blood stem cells as seed cells of tissue engineered meniscus and obtained satisfactory experimental results.

Other related research: In order to construct a tissue engineered meniscus that is more biomimetic to the natural meniscus structure and mechanical properties, some scholars have proposed the idea of cell coculture, the concept of partitioning the tissue engineered meniscus, and the high-density self-assembly culture method.

In the study of meniscus scaffold, the researchers conducted different studies mainly on scaffold materials, scaffold morphology, and 3D printing of scaffold.

A. Meniscus Scaffold Material

In terms of tissue engineered meniscus scaffold materials, they are mainly composed of synthetic polymer materials and biological materials related to extracellular matrix components. Synthetic materials mainly include PCL, polylactic acid (PLA), and PGA. The main advantage of synthetic materials is that they provide satisfactory biomechanical properties, unlimited supply, and strong plasticity. However, there are also disadvantages such as poor hydrophilicity, lack of biological activity, and possible induction of an inflammatory response. Biomaterials related to extracellular matrix components have broader application prospects than synthetic materials, because most of the extracellular matrix component-related biomaterials are biomimetic to the natural meniscus extracellular matrix, which can create a cell adhesion for seed cells, proliferation, or differentiation of the natural microenvironment. Today's extracellular matrix-related biomaterials are mainly composed of one or two extracellular matrix components, such as type I collagen scaffolds and type II collagen scaffolds, but these scaffolds do not fully mimic the microenvironment of natural meniscus cell growth. Some domestic institutions have also carried out research on meniscus scaffold, such as Lu Huading and Cai Daozhang, who used tissue-coated polyhydroxybutyrate hydroxyvalerate to construct tissue engineered meniscus scaffold. Zhu Yunli et al. used a scaffold constructed from autologous synovial mesenchymal stem cells, a small intestinal submucosa complex, to repair rabbit meniscus injury. Zhang Yefeng and Sun Lei explored the feasibility of forming decalcified bone matrix as a substitute for prosthesis after complete meniscus defect.

B. The Shape of the Meniscus Scaffold

Depending on the integrity of the meniscus scaffold morphology, the meniscus scaffold can be divided into partial meniscus scaffold and integral meniscus scaffold. Koller et al. constructed a partial meniscus scaffold for in vitro culture by adding polyethylene terephthalate to the hyaluronic acid/polycaprolactone scaffold. Baker et al. also

produced an oriented partial meniscus scaffold by electrospinning. Mandal et al. used silk fibroin to create an integral meniscus scaffold that mimics the natural meniscus shape and internal structure. Professor Stone et al. used a cow-derived Achilles ligament to make a collagen-copolymerized whole meniscus scaffold and was used in a multicenter clinical trial. After 1 year of implantation, biopsy results confirmed the presence of a similar tissue regeneration in the chronic meniscus lesion implantation group and was well integrated with the periphery of the host meniscus, and no improvement in clinical outcomes in patients with acute meniscus injury.

C. 3D Printed Meniscus Scaffold

3D printing has also been applied to the preparation of meniscus scaffold. For example, researchers at Columbia University Medical Center used biodegradable polycaprolactone as a material to create an alternative damaged meniscus using 3D printing technology. Part of the meniscus scaffold, the connective tissue growth factor and TGF-β3 in this scaffold can stimulate the migration and differentiation of stem cells inside the body, and promote the regeneration of meniscus tissue. In addition, Australian scientists used 3D printing technology to construct meniscus fiber hydrogel scaffold.

Many different types of growth factors are also used in the field of meniscal tissue engineering. bFGF can stimulate the proliferation of meniscal cells well. A group of studies have compared the effects of nine different growth factors on the proliferation of monolayer cultured meniscus cells—epidermal growth factor (EGF), bFGF, TGF-α, platelet-derived growth factor-AB (PDGF-AB), aFGF, TGF-β1, PDGF-AA, insulin-like growth factor-1 (IGF-1), and nerve growth factor (NGF). The results showed that bFGF, PDGF-AB, EGF, and TGF-α could stimulate cell proliferation, and bFGF had the strongest stimulation. The migration of cytokines to monolayer cultured cells has also been studied intensively. PDGF-AB and human growth factor (HGF) stimulate the migration of meniscus cells in three regions, EGF, IGF-1, interleukin-1 (IL-1), and bone morphogenetic protein-2. (BMP-2) can only stimulate cell migration in specific areas. In addition to studying the effects of cytokines on cell proliferation and migration, the effects of growth factors on the synthesis of extracellular matrices in seeds have also been extensively studied. The TGF-β family, as the most important stimulator of cartilage tissue engineering, also showed strong stimulation of the synthesis of the meniscus extracellular matrix (collagen and glycosaminoglycan) in the field of meniscal tissue engineering, and can also promote cell proliferation under monolayer culture conditions. Studies have shown that TGF-β1 can also promote the secretion of lubricating protein on the surface of cartilage. Maintenance of cell phenotypes and differentiation of cells into fibrocartilage are also important research directions for meniscal tissue engineering, although relatively few studies have been conducted in this area. However, some studies have shown that FGF-2 can reverse the phenotypic changes of meniscal cells well under monolayer cell culture conditions. Similarly, TGF-β1 has also been shown to promote phenotypic differentiation of meniscal fibrocartilage cells toward chondrocytes. Because meniscus cells are in different regions and their cell phenotypes are different, research in this area has great research prospects.

#### 9.7.2.6 Challenges of Cartilage Regeneration in Meniscus Engineering and the Direction of Future Conquest

There are also many challenges in tissue engineered meniscal seed cell research. If purely from the perspective of scientific research, autologous meniscus fibrocartilage cells will be the best tissue engineered meniscus seed cells. However, it is often difficult to obtain and there is dedifferentiation in subculture. Stem cells are more satisfactory seed cells for tissue engineered meniscus, but their clinical transformation applications are often limited by laws and regulations. Existing tissue engineered meniscus scaffold research also has some problems. For example, the existing meniscal scaffold material cannot completely simulate the natural microenvironment of meniscus cell growth. Most studies only focus on partial meniscus injury repair, and overall meniscus repair research is relatively few. The existing tissue engineered meniscus scaffolds are less concerned with the reconstruction of the collagen space structure in the meniscus, especially the 3D printed meniscus scaffold is mainly concentrated in the general form of bionics, while the internal space structure has less bionic reports. In the aspect of cytokine research, the specific mechanism of action is not particularly clear. On the one hand, it is necessary to study the effects of combining various cytokines in the future; on the other hand, the culture conditions (serum, monolayer culture, scaffold, and different forms) such as self-assembly also affect the effects of cytokines on cells. Future research may focus on nontraditional cytokines, including serum-derived phospholipids and lysophosphatidic acid (LPA).

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